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Contents

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CONTENTS

iii

ILLUSTRATIONS

PLATES

THE TOXIC INFLUENCE OF FLUORINE IN PHOSPHATIC FERTILIZERS ON THE GERMINATION
OF CORN

ISOLATION OF SOME BACTERIA WHICH OXIDIZE THIOSULFATE

THE AMPHOTERIC NATURE OF THREE COASTAL PLAIN SOILS: I. IN RELATION TO
PLANT GROWTH

KEIJIRO ASO

A NEW INSTRUMENT FOR SOIL SAMPLING

THE PREFERENTIAL UTILIZATION OF DIFFERENT FORMS OF INORGANIC NITROGEN IN THE DECOMPOSITION OF PLANT MATERIALS

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The experiments described in this paper were carried out with the object of discovering whether the organisms concerned in the decomposition of cellulosic matter exercise any preference for ammoniacal or nitric nitrogen when both forms are available to them in equal concentration.

It is now well established that practically any form of nitrogen will supply the needs of the fungi and bacteria which break down polysaccharide carbohydrates to humus and, in so doing, narrow the wide carbon-nitrogen ratio of the raw material.

Although almost any kind of nitrogen can be used, there are very marked differences in the effects brought about by the process of decomposition. For example, the maximum temperature reached, the color and physical condition of the end product, and the loss of elementary nitrogen sustained are all influenced by the form in which the nitrogen is supplied.

When the carbohydrate is decomposed in the soil it may easily happen that, in addition to the soil nitrate, much nitrogen is also present as ammonia. Such conditions are found when fresh unrotted farmyard manure is ploughed under, or when raw straw is returned to the soil together with a dressing of sulfate of ammonia.

The reactions of soil nitrogen to applications of strawy manure and to raw straw have been studied by many workers, and an extensive literature is devoted to this question. Only a few of these papers will be referred to here.

Niklewski (8) studied the effect of straw on the utilization of nitrogen in various compounds, including ammonium sulfate and sodium nitrate, by an oat crop grown in pots. Only one form of nitrogen was used in each pot, and attention was directed chiefly to effects of concentration and distribution in the soil. This worker, however, noted that straw had an unfavorable influence on the utilization of ammonium sulfate in lower concentrations but that the reverse was the case with similar concentrations of sodium nitrate. In other words, Niklewski found that nitrogen in the form of ammonium sulfate was rapidly immobilized by combination with straw so that the oat crop suffered, whereas sodium nitrate was, apparently, more completely assimilated by the crop in the presence of straw than in its absence. This last observation is rather surprising, but the results are in the same direction as those described in this paper.

1

Among others, Scott (10), Collison and Conn (2), Murray (6), and Gilbert and Pember (3) have all noted that the application of straw to soil results in a reduction of nitrate with consequent depression of crop yield. This effect could be overcome by addition of sufficient nitrogenous manure. The question of preference for any particular form of nitrogen is not discussed.

<div align="center">EXPERIMENTAL</div>

In the first instance the reactions of ammonia and nitrate with straw were tested alone, unmixed with soil. It is hoped to continue the experiments with nitrogen and straw in both light and heavy soils.

<div align="center">*Experiment 1*</div>

Air-dried chaffed wheat straw in lots of 20 gm. was well moistened by spraying. Sufficient solution of ammonium nitrate was added to each bottle to supply 1.0 per cent of nitrogen to the straw, half as ammonia and half as nitrate. The additional inorganic nitrogen immobilized as organic nitrogen by 100 gm. of any material in process of decomposition has been termed (9) the "nitrogen factor." As the nitrogen factor of normal wheat straw does not usually exceed 0.8 per cent there would then be a small excess of available nitrogen over and above that immobilized after rotting was complete.

Each bottle contained originally 18.5 gm. of dry matter as straw and 70 cc. water. The bottles were plugged with cotton wool and incubated at 35°C. Since the straw had 0.315 per cent nitrogen in dry matter, the bottles each contained 0.0582 gm. nitrogen in straw and 0.200 gm. nitrogen as ammonium nitrate, or 0.2582 gm. in all.

A bottle was removed at intervals over a period of 160 days for the determination of dry matter and ammoniacal, nitric, and total nitrogen. Ammonia was distilled from a sample made alkaline with magnesia, and nitrate was estimated in the residue by reduction with Devarda's alloy. These methods are known to give high values for both ammonia and nitrate when used on samples containing easily decomposed organic nitrogen. After the thirtieth day, for example, no blue coloration could be found with diphenyl benzidene (5), although the Devarda figure showed 0.04 per cent of nitric nitrogen in the wet sample. The color test is sensitive to one part in a million and it is certain that no nitrate was really present after the thirtieth day. The excess of apparent ammoniacal nitrogen comes, of course, in both cases, from the organic nitrogen and is not originally present as either ammonia or nitrate. More refined methods were used in the second series of experiments with the result that the values for both ammoniacal and nitric nitrogen after 28 days' incubation fell to about one-tenth of those found in the preliminary series.

The figures given in table 1 show clearly that from the start both forms of nitrogen are utilized by the organisms. During the first 15 days, however, when the fungal development is most active, there is a small but definite preference for ammonia over nitrate. On the eighth day, for example, 62 per cent

of the ammoniacal nitrogen but only 33 per cent of the original nitric nitrogen had been removed. In the later stages there is an approximately equal utilization of both forms, but it is very important to remember that the gradual reduction of nitric nitrogen is due to loss as elementary nitrogen as well as to assimilation, whereas the ammoniacal nitrogen is all immobilized if not supplied in excess of the nitrogen factor of the cellulosic base. This difference is more clearly shown in the second series of experiments.

Experiment 2

In this experiment four different sets of bottles, each containing 20 gm. of air-dry oat straw, were incubated for 56 days. Nitrogen was supplied as (a) ammonium carbonate, (b) ammonium nitrate, (c) sodium nitrate and (d) a mix-

TABLE 1

Wheat straw rotted with ammonium nitrate at 35°C.

Calculated on 100 gm. of original dry straw

DAYS	LOSS OF DRY MATTER	NITROGEN PRESENT AS			NITROGEN FACTOR
		NH$_3$	NO$_3$	Total	
	gm.	gm.	gm.	gm.	per cent
0	....	0.54	0.54	1.40	....
1	Nil	0.42	0.55	1.37	0.09
3	3.9	0.44	0.50	1.43	0.18
8	20.4	0.21	0.36	1.26	0.40
15	25.6	0.28	0.31	1.32	0.43
22	29.1	0.23	0.28	1.32	0.52
30	36.2	0.19	.....	1.09	0.56
36	36.0	0.19	0.21*	1.28	0.59
136	56.2	0.13	0.22*	1.14	0.74
160	48.8	0.11	.....	1.17	0.78

* These figures are certainly too high for the reason explained in the text.

ture of (a) and (c). As 0.20 gm. of nitrogen was added to each bottle there was present at the start an excess over the amount expected to be immobilized in rotting. After the 20 bottles of this series had all been analyzed, a final set of five bottles of oat straw was incubated to which 0.32 gm. nitrogen, i.e., more than double the nitrogen factor, was added as ammonium nitrate. The object of this experiment was to determine whether a supply of nitrogen in the form of either ammonia *or* nitrate sufficient to complete the reaction without calling on the other would influence the result.

Ammoniacal and nitric nitrogen were estimated as follows:

Ammonia. The method was devised by Nichols and Foote (7) for the estimation of free ammonia in sewage and trade wastes. A phosphate buffer solution was made up by dissolving 14.3 gm. KH$_2$PO$_4$ and 90.15 gm. of K$_2$HPO$_4 \cdot 3$H$_2$O in distilled water made up to 1 liter. About 10 gm. of the wet manure were

distilled from 300 cc. of water with 25 cc. of the phosphate buffer solution. In this way the separation of the free ammonia from the organic nitrogen was conducted at a pH of about 7.4, which was maintained constant by the phosphate buffer.

Nitrate. This was estimated by Bengtsson's method (1). Ten grams of the wet manure were extracted with 300 cc. of distilled water in fractions of 50 cc. Each fraction was allowed 10 minutes' extraction with occasional stirring. The supernatant solution was decanted through a cotton wool plug filter. This process was repeated until the extract no longer gave a blue coloration with diphenyl benzidene. The suspended organic matter and colloids were precipitated with a few drops of H_2SO_4, and the solution was warmed. The settled matter was filtered off on a Buchner funnel under suction. The filtrate was made alkaline to litmus with NaOH and evaporated down to about 30 cc. After being made up to 200 cc., including 25 cc. of 10 per cent NaOH, the filtrate was again evaporated to 30 cc. By this time any ammonia nitrogen originally present as such and any produced from organic nitrogen in the extract had been volatilized. Usually one evaporation was sufficient. The residue was finally distilled from about 300 cc. of water and Devarda's alloy and the ammonia absorbed in standard acid as usual.

The effect of the improved methods of estimating ammonia and nitrate is clearly seen in table 2. After the fourteenth day of the experiment the amount of ammoniacal nitrogen is about one-tenth of that found in the first experiment (table 1) after an equal period of rotting. The nitric nitrogen is also reduced to a similar extent. Comparison is possible only with the ammonium nitrate series in experiment 2, but the values for inorganic nitrogen are consistently low in the later stages of all the series in which other nitrogen compounds were under test.

The preference of the organisms for ammoniacal nitrogen in the first 14 days shown by the preliminary experiment is confirmed in the two series of experiment 2 in which the nitrogen was supplied as ammonium nitrate and as a mixture of ammonium carbonate and sodium nitrate. Further, if the figures for series (a) ammonium carbonate, are compared with series (c) sodium nitrate, it will be noticed that on the seventh day the ammoniacal nitrogen has been reduced by 96 per cent but the nitric nitrogen by only 59 per cent. By the fourteenth day the maximum amount of nitrogen had been converted to protein in all three series where nitrate was originally added, after that the nitrogen factor declines and the residual inorganic nitrogen is usually less than 0.10 per cent of the dry matter in the sample. On the other hand when the nitrogen is supplied entirely as ammonia the nitrogen factor remains at a maximum up to the fifty-sixth day, and the percentage of nitrogen in the final product is higher than in any other samples of this experiment. At this stage the ammonification of protein has begun, so that rather more ammonia is found on the fifty-sixth than on the twenty-eighth day, irrespective of whether the nitrogen was originally supplied as ammonia or nitrate.

TABLE 2

Oat straw rotted with different nitrogen compounds at 35°C.

Calculated on 100 gm. of original dry straw

DAYS	DRY MATTER	NITROGEN PRESENT AS			LOSS OF N	NITROGEN FACTOR
		NH₃	NO₃	Total		
	gm.	gm.	gm.	gm.	per cent	per cent
(a) Ammonium carbonate						
0	100.0	1.15	....	1.64	....	....
3	100.0	0.84	....	1.35	17.8	....
7	94.2	0.05	....	1.27	22.7	0.28
14	70.7	0.04	....	1.35	18.0	0.81
28	60.1	0.03	....	1.25	23.9	0.72
56	43.6	0.06	....	1.40	14.8	0.85
(b) Ammonium nitrate						
0	100.0	0.57	0.57	1.64	....	....
3	100.0	0.32	0.49	1.27	22.5	....
7	89.1	0.05	0.15	1.21	26.9	0.51
14	65.1	0.03	0.04	1.34	18.6	0.78
28	57.0	0.02	0.01	1.11	32.2	0.59
56	40.4	0.05	0.03	1.15	29.9	0.58
(c) Sodium nitrate						
0	100.0	....	1.15	1.64	....	....
3	100.0	0.03	1.01	1.28	22.2	....
7	92.2	0.03	0.47	1.11	32.2	0.11
14	76.3	0.02	0.15	1.14	30.5	0.48
28	62.6	0.02	0.02	1.01	38.4	0.47
56	47.4	0.04	0.02	0.92	44.4	0.36
(d) Ammonium carbonate and sodium nitrate						
0	100.0	0.57	0.57	1.64	....	....
3	100.0	0.36	0.48	1.31	19.7	....
7	86.3	0.04	0.12	1.09	33.6	0.44
14	73.9	0.03	0.03	1.31	20.2	0.77
28	62.0	0.02	0.01	1.17	28.7	0.64
56	43.6	0.04	0.02	1.08	34.6	0.52
(e) Ammonium nitrate (higher concentration)						
0	100.0	0.93	0.93	2.27	....	....
3	96.1	0.46	0.44	1.32	41.7	....
7	92.7	0.30	0.28	1.32	41.7	0.29
14	73.2	0.06	0.03	1.39	38.8	0.86
28	61.7	0.05	0.04	1.47	35.8	0.94

As the loss of nitrogen during the rotting of the various samples of straw was often considerable and this question is of practical importance, the conditions which gave rise to the greatest loss are worth noting. Throughout the whole experiment, with the exception of the final series with a higher concentration of ammonium nitrate, nitrogen was added in excess of the normal nitrogen factor of the oat straw (0.85) to the extent of 25 per cent. This amount is therefore the greatest loss to be expected if no inorganic nitrogen remained unassimilated. Allowing for the ammonia not volatilized, this result is substantially found in series 2 (a) in which ammonium carbonate was used. In the other series, all containing nitrate, there is a greater loss of nitrogen which is at a maximum of 44 per cent in series 2 (c) (sodium nitrate). Further, when nitrate is present the nitrogen factor is always lower than when the nitrogen is supplied entirely as ammonia. There seems to be some cause which inhibits the growth of those organisms, probably fungi, responsible for the high proportion of protein found when ammonia is the only source of nitrogen. This effect was most marked in series 2 (c) (sodium nitrate). There the nitrogen factor is only a little more than one-half the normal value. The trouble is not due to shortage of mineral nitrogen, for plenty of unused nitrate is present on the fourteenth day when the nitrogen assimilated was at a maximum. Possibly the alkalinity of the rots with $NaNO_3$ (pH 9–10) may be partly responsible, but the effect is noticeable in series 2 (b) (ammonium nitrate), where the reaction was approximately neutral. Nitrate does not check the oxidation of the carbohydrates—only the development of protein. Indeed the greatest loss of dry matter in the whole series of experiments, almost 60 per cent, was found in series 2 (b) (ammonium nitrate).

Very soon after the first trials of artificial farmyard manure had been made (4) it was recognized that from the economic point of view nitrate was one of the least desirable forms of nitrogen for that purpose. Apart from its high cost per unit of nitrogen the possibility of denitrification must always be present. The experiments described in this paper show clearly how large these losses of nitrogen may be even in bottles where no leaching can occur. Manures made with nitrate nitrogen, in whole or in part, are inferior to those made with ammonium carbonate, as judged by the total nitrogen in the final dry matter and by the nitrogen immobilized per 100 gm. of original straw (nitrogen factor). In the case of sodium nitrate used alone the comparison is particularly unfavorable to nitrate.

Besides richness in protein the yield of manure is also important, and in this respect there is little difference whatever form of nitrogen is used. The loss of dry matter after 28 days is about 40 per cent in all cases. At this stage, equivalent to about four months in a manure heap under average conditions, the products are generally at their best for application to the soil.

When strawy unrotted farmyard manure is applied to a soil containing a reserve of nitrate the organisms have a choice of using either the ammoniacal

nitrogen in the urine-saturated straw or calling on the soil nitrate. These experiments suggest that the ammonia will at first be utilized but that some nitrate will also be built up into protein in spite of the fact that there is more than sufficient ammoniacal nitrogen to satisfy all requirements. At the same time some of the nitrate nitrogen will probably be lost as elementary nitrogen.

A similar series of changes will no doubt occur when raw straw is ploughed into the soil along with sufficient available nitrogen to avoid the depression of crop yield which otherwise follows the immobilization of the soil nitrates. The straw will draw mainly on the ammonia, but some nitrate will also be taken with the usual associated loss of elementary nitrogen.

It is hoped to make some experiments with straw and ammonium-nitrate mixtures in both light and heavy soils to test the validity of the hypotheses submitted.

SUMMARY

When straw is in contact with both ammoniacal and nitric nitrogen in equal initial concentration under conditions favorable for decomposition there is a definite preference by the organisms concerned in the earlier stages of breakdown for ammonia rather than for nitrate.

After 14 days at 35°C. unassimilated inorganic nitrogen is about equally divided between ammonia and nitrate. There is then no apparent preference for either form.

When nitrogen is supplied wholly or partially as nitrate the nitrogen factor calculated after rotting is complete is always lower than when ammonia is used.

The loss of nitrogen is always greatest when nitrate is present. Sodium nitrate and ammonium nitrate lost 30 and 15 per cent more nitrogen, respectively, than ammonium carbonate in equal original concentration.

As a result of this loss of, presumably, elementary nitrogen from nitrate, the relative assimilation of ammonia may be greater than the figures indicate, since the drop in nitrate includes both the nitrogen lost as well as that assimilated.

Some practical applications of these results are discussed.

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RELATIVE AMOUNTS OF·CALCIUM CARBONATE AND MAGNE-SIUM CARBONATE IN SOME MINNESOTA SUBSOILS

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About three-fourths of the improved farm land in Minnesota lies on the Des Moines lobe of the Late Wisconsin drift, or upon outwash or lacustral deposits derived from it. A notable feature of this drift is its calcareousness. In most places on its till plains and clayey moraines carbonates have been leached out to a depth of 2 to 4 feet but below this they constitute from 6 to 30 per cent of the fine earth (5, p. 81; 7, p. 194). Less commonly they are abundant even in the second foot. Fragments of limestone form a large proportion of the gravel and pebbles of the unleached subsoil (4, p. 14).

It is of interest to learn whether the carbonate is present chiefly as calcite or as dolomite in these calcareous subsoils, in which a considerable portion of the roots of some of the most important farm crops develop. The subsoil exposed in drainage ditches provides the most economical source of liming material for nearby lime-deficient soils in some places in northern Minnesota. Analyses showing how the carbonates in the fine earth, the portion passing a 2-mm. sieve, are divided between calcium and magnesium have not been published in this country or in Canada, in so far as we have been able to ascertain.

Soil analyses, as ordinarily made, do not give the desired information, because, although the percentages of lime, magnesia, and carbon dioxide are reported, the lime and magnesia have in part been derived from the decomposition of silicates and from the exchange complex, no matter whether the usual acid extraction methods or those of rock analysis have been used. As a preliminary investigation we have determined the amounts of calcium and magnesium present as carbonates in 25 samples of subsoil of known CO_2 content, selected from collections made in connection with various earlier studies.

SAMPLES ANALYZED

The sets of samples from which the selection was made had been taken to represent various soil profiles, usually to a depth of 6 feet. In most cases the division of the profile had been by arbitrary depths, as second foot, third foot, etc., but in some, according to the soil horizons. Most of the samples represent the uppermost section of the profile that was found to effervesce distinctly when treated with dilute hydrochloric acid. From five of the profiles a sample from a greater depth has been included. All are from sites where the natural drain-

TABLE 1

Relative amounts of calcium carbonate and magnesium carbonate

NUMBER	LOCALITY	SOIL TYPE	DEPTH OF SECTION	MOISTURE EQUIVALENT	CO₂ CONTENT		CARBONATE IN SAMPLE			PROPORTION OF CARBONATES PRESENT AS		RATIO CaCO₃ mols / MgCO₃ mols
					Determined	Computed	CaCO₃	MgCO₃	Total	Dolomite CaCO₃ MgCO₃	Calcite CaCO₃	
			inches		per cent	per cent	per cent	per cent	per cent	per cent	per cent	
1	Mizpah	Beltrami silt loam	25–36	21.1	4.30	4.09	6.04	2.74	8.78	68.3	31.7	1.87
2	Mizpah	Beltrami silt loam	61–72	18.4	6.85	6.86	10.15	4.60	14.75	68.3	31.7	1.87
3	Taylors Falls	Miami loam	61–72	18.8	3.15	3.51	5.00	2.51	7.51	73.1	26.9	1.68
4	Hamel	Hayden loam	37–48	26.7	4.55	4.86	7.33	3.14	10.47	65.7	34.3	1.98
5	Hamel	Hayden loam	61–72	22.2	7.55	7.65	11.85	4.70	16.55	62.2	37.8	2.13
6	Renville	Webster silty clay loam	25–36	27.3	8.90	8.84	14.81	4.47	19.28	49.7	50.3	2.81
7	Renville	Webster silty clay loam	61–72	26.3	8.65	8.61	13.00	5.56	18.56	65.7	34.3	1.97
8	Canby	Barnes silt loam	25–36	23.3	7.65	7.60	14.29	2.52	16.81	32.8	67.2	4.80
9	Canby	Barnes silt loam	61–72	22.0	7.75	7.75	13.70	3.31	17.01	42.5	57.5	3.50
10	Morristown	Fargo silt loam	13–24	35.6	2.70	3.09	5.54	1.26	6.80	40.5	59.5	3.75
11	Morristown	Fargo silt loam	25–36	35.1	3.70	3.70	5.82	2.18	8.00	59.6	40.4	2.27
12	Albert Lea	Clarion loam	25–36	25.6	2.20	2.29	3.14	1.76	4.90	78.6	21.4	1.51
13	Wells	Webster clay loam	25–36	27.0	5.21	5.21	9.25	2.20	11.45	42.0	58.0	3.57
14	Wells	Webster clay loam	25–36	27.7	6.60	6.09	11.25	2.20	13.45	35.9	64.1	4.34
15	Fairmont	Clarion silt loam	25–36	21.8	7.60	6.97	12.17	3.12	15.29	44.7	55.3	3.30
16	Fairmont	Clarion silt loam	25–36	22.0	7.70	7.69	13.60	3.29	16.89	42.7	57.3	3.49
17	Jackson	Webster silty clay loam	25–36	24.6	6.15	6.07	10.45	2.84	13.29	46.9	53.1	3.10
18	Worthington	Clarion silt loam	25–36	23.7	3.70	3.69	6.50	1.60	8.10	43.4	56.6	3.45
19	Worthington	Clarion silt loam	25–36	26.2	6.50	6.57	11.58	2.84	14.42	43.1	56.9	3.44
20	Adrian	Clarion silt loam	25–36	24.6	4.00	3.66	5.59	2.30	7.89	63.9	36.1	2.05
21	Adrian	Clarion silt loam	25–36	17.0	4.10	3.96	6.45	2.15	8.60	54.7	45.3	2.54
22	Star Island	Cass Lake fine sand	37–72	2.5	0.50	0.46	0.75	0.25	1.00	54.7	45.3	2.54
23	Star Island	Cass Lake fine sand	49–72	2.6	1.80	1.40	2.45	0.61	3.06	43.6	56.4	3.37
24	Star Island	Cass Lake fine sand	61–84	2.5	0.80	0.88	1.55	0.42	1.97	46.6	53.4	3.01
25	Star Island	Cass Lake fine sand	49–72	2.4	1.36	1.34	2.20	0.71	2.91	53.4	46.6	2.62

age was good or moderately so, all were free of chlorides and sodium carbonate, while water-soluble sulfates, including gypsum, were either absent or present only in traces. Pebbles were excluded from all.

The samples were taken by means of augers. Of the 25, 21 are from soil types developed on till plains or clayey moraines, 19 on the Late Wisconsin drift and 2 from Adrian on the Iowan (4, p. 29). All these are composites made from a number of individual samples. In the case of Nos. 1 to 9 each is a composite from 15 sites, 5 sites about 10 yards apart in each of three fields a mile or more apart. Nos. 10 to 21 each represent a single field, Nos. 10 and 11 being taken from 5 sites and Nos. 12 to 21 from 10. The remaining four samples, Nos. 22 to 25, are each from a single boring on Star Island, part of a sandy outwash plain derived from the Late Wisconsin drift (1, p. 282). None are from within the limits of glacial Lake Agassiz (fig. 1).

<div align="center">METHOD OF ANALYSIS</div>

The sample, freed of rock fragments exceeding 2 mm. in diameter, was ground in an iron mortar to pass a 100-mesh sieve. A weighed portion, 5 or 10 gm. according to the richness of the sample in carbonates, was placed in a beaker with 50 to 100 cc. water and treated with the exact volume of 2 N HCl corresponding to its CO_2 content. The mixture was stirred frequently, allowed to stand over night, next morning stirred again and transferred to a Büchner funnel. The residue was washed free of chloride, the filtrate was made up to definite volume, and, in an aliquot, calcium and magnesium were determined by the usual methods.

To make sure that the carbonates had been entirely decomposed the residue was treated with hot dilute hydrochloric acid, but in no case was effervescence detected. Very little decomposition of the silicates was caused by the hydrochloric acid. With three samples the amount of SiO_2 in the filtrate was determined and in each found to be less than 0.10 per cent.

In computing the percentages of $CaCO_3$ and $MgCO_3$ it has been assumed that all of the CaO and MgO extracted by this acid treatment were present as carbonates. The amount of CO_2 corresponding to these is reported in the seventh column of table 1 and agrees closely with that actually determined by the use of an absorption tower and titration method. Thus in the case of sample No. 1, in which 4.30 per cent CO_2 was found by absorption, the hydrochloric acid dissolved 3.38 per cent of CaO and 1.31 of MgO, corresponding to 2.66 and 1.43 per cent, respectively, of CO_2, or to a total of 4.09 per cent.

<div align="center">DISCUSSION</div>

As indicated by the moisture equivalents, reported in the fifth column of table 1, all the samples are of fine or moderately fine texture except the four from Star Island. The total carbonate content ranges from 1.00 to 19.28 per cent, and dolomite forms from a fifth to two-thirds of this, assuming that the whole of the magnesium carbonate is present as dolomite, $CaMg(CO_3)_2$.

Accordingly the ratio of $CaCO_3$ molecules to $MgCO_3$ molecules ranges from 1.51 to 4.80, whereas for dolomite the ratio is 1.00. From five localities samples from two depths were analyzed; at two, Mizpah and Hamel, the ratios are similar—1.87 vs 1.87 and 1.98 vs. 2.13, but at the three others the ratios are rather widely divergent, at Renville 2.81 vs. 1.97, at Canby 4.80 vs. 3.50, and
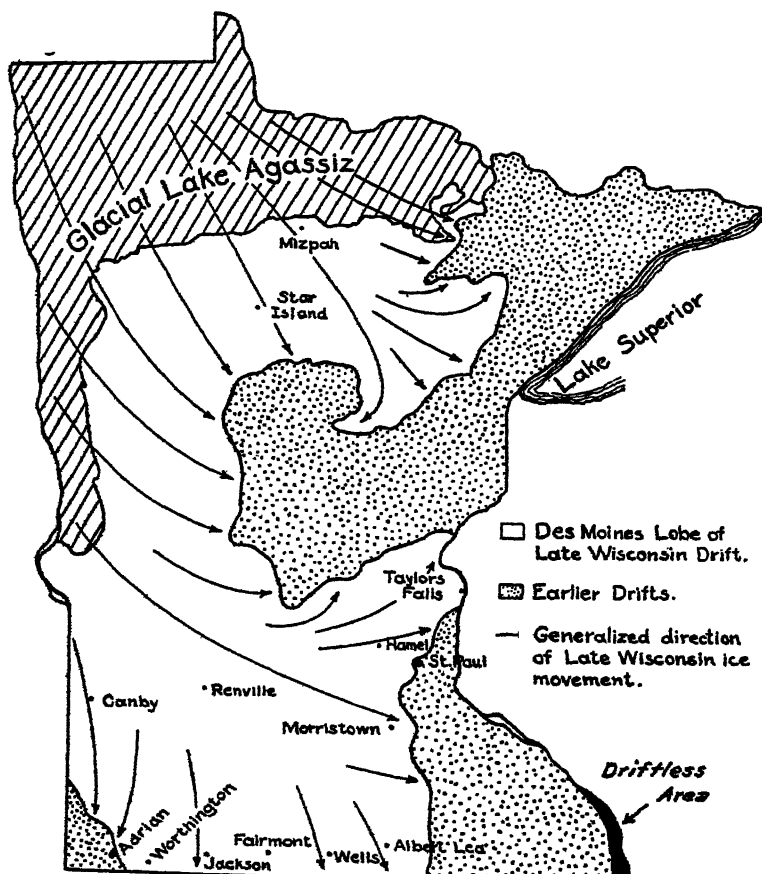
FIG. 1. MAP OF MINNESOTA SHOWING EXTENT IN THE STATE OF THE DES MOINES LOBE OF THE LAST GLACIAL ICE SHEET, DIRECTIONS OF ICE MOVEMENT AND PORTION COVERED BY LAKE AGASSIZ

After Leverett (4, p. 4). The locations of the areas sampled are indicated

at Morristown 3.75 vs. 2.27. Samples from the same depth but from fields a few miles apart show similar ratios at Fairmont, 3.30 vs. 3.49 and at Worthington, 3.44 vs. 3.45, but at Adrian the ratios are more divergent, 2.05 vs. 2.54. At Wells the ratios for samples from the same depth on different parts of the same field are even more dissimilar, 3.57 and 4.34. The four samples from Star Island, taken from sites less than a mile apart, show a range from 2.54 to 3.37.

In no sample does the ratio approach that in dolomite. In the heavy subsoils the supply of magnesium carbonate is very large, considering possible future crop needs of available magnesium, varying from 25 to 100 tons per acre foot.

Western Manitoba was the glacial gathering ground of the carbonates of the Late Wisconsin drift (4, p. 3–4), limestone rocks in Minnesota being confined to the southeastern corner of the state and to a small area in the extreme northwestern corner immediately adjacent to Manitoba (4, p. 14). The calcareous material of this drift was in part brought from Manitoba by the last ice sheet and in part derived from the older, Kansan and Nebraskan, drifts, over which it passed in its southeastward advance (fig. 1), both of which had

TABLE 2

Composition of Manitoba limestones

From reports of Goudge (2, p. 4–8; 3, p. 105) and Wallace and Greer (8, p. 33–52)

NUMBER	LOCALITY	CaCO₃	MgCO₃	RATIO CaCO₃ mols. / MgCO₃ mols.	NUMBER	LOCALITY	CaCO₃	MgCO₃	RATIO CaCO₃ mols. / MgCO₃ mols.
		per cent	per cent				per cent	per cent	
1	Sifton Narrows	53.80	45.55	1.00	17	Tyndall	84.46	13.57	5.24
2	Garson	51.08	41.90	1.02	18	Tyndall	85.92	12.34	5.85
3	Stony Mountain	53.79	44.37	1.02	19	Garson	89.67	8.40	8.97
4	Cormorant Lake	53.21	43.26	1.03	20	Oak Point	89.14	7.82	9.57
5	Stonewall	54.82	44.84	1.03	21	Oak Point	90.00	7.19	10.51
6	Stonewall	53.51	42.91	1.05	22	Oak Point	89.08	6.81	10.98
7	Garson	48.11	37.95	1.06	23	Garson	94.02	4.33	18.26
8	Broad Valley	55.35	43.26	1.07	24	Oak Point	95.44	2.78	28.84
9	Broad Valley	55.05	42.75	1.08	25	Winnipegosis	94.80	2.12	37.62
10	Stony Mountain	52.63	40.99	1.08	26	Tyndall	97.09	1.68	48.54
11	Stony Mountain	56.25	42.17	1.12	27	Spearhill	96.56	1.42	57.11
12	Stonewall	57.22	41.12	1.17	28	Spearhill	96.11	1.39	58.02
13	Tyndall	68.98	28.16	2.06	29	Spearhill	96.40	1.24	65.13
14	Garson	71.03	23.35	2.56	30	Oak Point	97.26	0.84	97.26
15	Hecla Island	76.21	16.10	3.97	31	Winnipegosis	98.67	Trace	.....
16	Garson	82.12	16.25	4.24					

had the same gathering ground and were similarly calcareous, limestone form- ing usually 50 per cent or more of the pebbles in these (4, p. 14). Samples 20 and 21 were taken from the Iowan drift, which is somewhat older than the Late Wisconsin but very much younger than the Kansan and appears to have had the same gathering ground.

In Manitoba there are two large areas of limestone, both of Palæozoic age, one bordering Hudson Bay and the other exposed from Winnipeg northwest- ward to north of The Pas as a strip about 100 miles wide (2, p. 2). Only the latter has influenced the composition of the Des Moines lobe of the Late Wis- consin drift. It includes the basins of Lakes Winnipegosis and Manitoba and

the western half of Lake Winnepeg; in it three geological periods are repre-
sented, Ordovician, Silurian, and Devonian.   Although high-calcium lime-
stones have been found in various places, the rocks are prevailingly dolomitic.
We have found published analyses of 31 samples of Manitoba limestones
and from these have prepared table 2, arranging the data in order of approach
to pure dolomite.   In 12 of the samples the ratio is below 1.18; in 3, between
2.0 and 4.0; and in 8 it is above 25.0, the magnesium carbonate in these being
less than 3.00 per cent.   The average ratios for the 25 subsoil samples is 2.84,
which is far below that in the high-calcium limestones but much above that in
the dolomite samples.

It should be pointed out that the method used in collecting the soil samples,
the making of composites from a large number of borings a considerable
distance apart, prevents the analyses revealing whether there may exist sharp
variations in the ratio from level to level in certain calcareous profiles, such as
in the upper part of the zone of carbonate accumulation in pedocals (5, p. 79;
6, p. 18).   To answer this question the samples should be taken in short sec-
tions from individual profiles, as was done by McMiller in a study of the total
carbonate content of some soils in western Minnesota (5).

SUMMARY

The relative amounts of calcium carbonate and magnesium carbonate were
determined in 25 calcareous subsoils from different parts of Minnesota, most
of them from fine-textured soil types developed upon till plains and clayey
moraines of the Late Wisconsin drift.   The ratio of $CaCO_3$ molecules to $MgCO_3$
molecules ranged from 1.51 to 4.80, with an average of 2.84.   If it is assumed
that all the magnesium carbonate is present in the form of dolomite the latter
constitutes on the average a little more than half of the total carbonates, the
minimum found being 32.8 and the maximum 78.6 per cent.   In the heavy
subsoils the supply of magnesium carbonate is very high in comparison with
crop needs of available magnesium, varying from 25 to 100 tons per acre-foot.

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TOXICITY OF MANGANESE TO TURKISH TOBACCO IN ACID KENTUCKY SOILS[1]

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In studies on frenching of tobacco, for several years Turkish tobacco was grown in the greenhouse on soils from some of the Kentucky soil experiment fields. An abnormal condition developed in the plants on soil from certain plots that had received nothing except manure. This began as a chlorosis in the tips of the leaves, later spread over the leaves and, when severe, produced numerous dead areas and much leaf distortion. This condition evidently was not due directly to H-ion concentration, since field superphosphate treatments more or less prevented it; the pH of the soils was no lower than that of water cultures in which plants were uninjured; and the degree of injury correlated only in a general way with pH of the soils. It seemed probable that the injury was caused by aluminum or manganese—more likely the latter. Jacobson and Swanback (2) reported manganese to cause what appeared to be a similar abnormal condition in tobacco grown on certain acid Connecticut soils. Many Kentucky soils have been found to contain considerable amounts of manganese (3, 11). In a preliminary water culture experiment in 1931, manganese additions produced a condition in Turkish tobacco very similar to that in the plants in the soils.

The work reported in this paper was done to determine the cause of this abnormal growth condition. It included the growing of Turkish tobacco in the greenhouse in soils from the experiment fields and in water cultures; analysis of soil leachates; analysis of the plants grown.

EXPERIMENTAL RESULTS

Growth of Turkish tobacco in the greenhouse in soils from the experiment fields

The soils used, certain information about them, and the plan of the soil culture experiments are shown in table 1. Eighteen hundred grams of air-

15

dry soil were weighed into one-half-gallon glazed earthenware jars. Glass tubes 1.5 inches in diameter extended from above the soil to the bottom of the jars to facilitate watering and aeration. Distilled water was added from time to time to maintain a moisture content of 25 per cent of the dry soil. Two Turkish tobacco plants were set in each jar on January 3, 1933.

TABLE 1

Soils used, field data, and set-up of soil cultures

SOIL EXPERIMENT FIELD	YEAR ESTAB- LISHED	DATE SOILS OBTAINED†	PLOT NO.	FIELD TREATMENT* TOTAL POUNDS PER ACRE	SOIL CULTURE JAR NO.	KIND OF SOIL
Berea	1913	Oct. 1923	203	M 20,810	1, 2	Gray silt loam
			302	M 12,000 L 6,000	3, 4	Natural drainage poor
Greenville	1913	Oct. 1923	310	M 12,000	5, 6	Tilsit silt loam
			307	M 12,000 L 14,000 SP 2,400	7, 8	
Mayfield	1913	Oct. 1923	307‡ 507‡	M 24,290 M 20,810	9, 10	Memphis silt loam
		1928	202	M 36,180 L 8,000	11, 12	
		1928	204	M 31,560 SP 2,600	13, 14	
Campbellsville	1919	Aug. 1928	713	M 10,000	15, 16	Silt loam soil de- rived from lime- stone and shale
		May 1930	203	M 19,360 L 8,000	17, 18	

* M = manure; L = limestone; SP = superphosphate. (In this paper in referring to plot treatments, the manure treatments will be omitted, since they were made to all plots. Plots receiving manure only will be referred to as check plots.)

† These soils had been brought in for various purposes.

‡ Mixture of equal parts of the soil from the two plots.

Six days after the plants were set chlorosis had developed in the Berea and Greenville check jars, 1, 2, 5, 6. In two more days, it had developed in the Mayfield check jars 9, 10; in the manure-phosphate jars 13, 14; and in the Campbellsville check jars 15, 16. On January 18, chlorosis had continued to develop in plants in the Berea and Greenville check jars (nos. 1, 2, 5, 6) but was less in the other plants. On February 7, the chlorotic condition had practi- cally disappeared from all the plants except those in the Berea and Greenville check jars. Injury was very severe in these plants. The leaves were pitted with

brown, irregular spots and were greatly distorted. At no time was chlorosis present in plants grown on limed soils. (See plates 1, 2, and 3.)

Effect of manganese and aluminum on Turkish tobacco grown in the greenhouse in water cultures

Turkish tobacco plants were grown in three experiments in water cultures to which were added varying amounts respectively, of manganese and aluminum. Pint Mason jars were used. One plant was grown in each jar. The culture solution contained the following salts per liter: 1.00 gm. $Ca(NO_3)_2$, 0.25 gm. K_2SO_4, 0.20 gm. $CaH_4(PO_4)_2$, 0.10 gm. $MgCl_2$, 0.001 gm. H_3BO_3, and 1.0 cc. of a saturated solution of ferric tartrate. The solutions were changed daily to maintain the concentration of the various ions. The roots of the plants were washed with distilled water each time the solutions were changed.

In the first experiment plants were set on March 18, 1933, and allowed to grow in the nutrient solution until March 30. At this time the plants had formed new roots and were making good growth. Manganese and aluminum treatments were then added to duplicate jars. In treatment 1, the pH was maintained at 6.2; in treatment 2, at 4.5.[3] Treatments 3 to 6, inclusive, represented additions of 1, 2, 4, and 6 p.p.m., respectively, of aluminum as $Al_2Cl_6 \cdot 12H_2O$. Treatments 7 to 11, inclusive, represented additions of 4, 6, 8, 10, and 12 p.p.m., respectively, of manganese as $MnCl_2 \cdot 4H_2O$. The pH was maintained at 4.5 in these aluminum and manganese-treated cultures. Phosphorus was omitted from the aluminum cultures to prevent precipitation of aluminum phosphate. To supply phosphorus, these plants were alternated daily between the aluminum-treated culture and a similar culture receiving phosphorus but containing no aluminum, a modification of the method of McLean and Gilbert (6).

The injurious effects of all the aluminum treatments were observable within 3 days. The root tips blackened. Roots no longer lengthened but thickened to twice their normal size. Numerous new roots formed above the level of the culture solution but died upon reaching the solution. New roots also formed in the culture solution, but made only small growth before dying. The toxic effect of aluminum on plant roots has been noted in other crops (6, 10, 12). Top growth was reduced, particularly in size of stem and extent of leaf spread. Late in the experiment a slight tipburn developed in the lower leaves of the plants treated with 4 and 6 p.p.m. of aluminum. At no time did any chlorosis develop in the leaves of these plants or any symptoms appear similar to those in the plants in the unlimed soils.

The manganese-treated plants made normal growth. No injury developed in the tops or the roots within a period of 21 days, when the experiment was discontinued.

In the second experiment the cultures contained 15, 20, 25, 30, 35, 40, 45, 50,

[3] The pH of the nutrient solution as made up was 6.2. pH 4.5 was obtained by the addition of HCl.

and 55 p.p.m. of manganese, respectively. The treatments were not in dupli-
cate. The plants were started in the culture solutions at the same time as
those in the first experiment, March 18, 1933. The manganese additions
were made on April 15. At this time the plants were 4 to 5 inches high and
had good root systems. Chlorosis developed in all the plants within 6 days
after treatment and increased in severity with increase in manganese addition.
Fifteen p.p.m. of manganese produced only a yellowing in the tips and margins
of the leaves. With the larger manganese additions, most of the leaf tissue
was affected, and there was considerable development of brown chlorotic areas
and leaf distortion. The appearance of the plants was very similar to that of
the abnormal plants in the soils. The roots were not affected (roots of the
plants grown in the soils were unaffected). Funchess (1) reports that manga-
nese injury is very largely confined to the tops of the plants. From observa-
tion the manganese treatments did not reduce plant growth in the 13 days the
experiment was continued. Plate 4, figure 1, shows the effect of manganese on
the plants in these cultures.[4]

In a third experiment the aluminum and manganese additions were made at
the time the plants were set. The aluminum treatments were 0.5, 1.0, and 2.0
p.p.m., respectively, and the manganese treatments 15, 20, and 25 p.p.m.

There was no observable effect from 0.5 p.p.m. of aluminum. The other
aluminum treatments affected the plants as in first experiment. The three
manganese treatments affected the plants as in the second experiment. Fif-
teen p.p.m. manganese produced slight chlorosis. The other two treatments
produced considerable chlorosis and some brown spots.

Effect on manganese toxicity of phosphorus additions to both soil and water cultures

As stated in the introduction, it had been observed that field phosphate
treatments largely prevented the development of the abnormal condition in
the plants. The soils producing the chlorotic plants in this study were phos-
phate deficient. On March 1, 1933, 1 gm. of $Na_3PO_4 \cdot 12H_2O$ was added to
each of jars 2 and 6, Berea and Greenville check soils, respectively. The plants
in jar 2 averaged 3 inches high; those in jar 6, 2.5 inches high. Growth and
recovery soon began. By the end of March the plants in the Greenville soil
(jar 6) had almost entirely recovered and the plants in the Berea soil (jar 2)
had largely recovered. The phosphate treatments were repeated on April 1.[5]

[4] Since this paper was written barley and red clover plants have been grown by the writer
in water cultures containing manganese in concentrations of 5 to 40 p.p.m.

Black spots appeared on the tips of the barley leaves in all cultures 4 days after treatment.
These spots later appeared over the entire blade and with the heavier manganese concentra-
tion also on the stalk. However, no yellow chlorosis developed.

The red clover plants developed yellow chlorosis on the leaf margins of all the new leaves
within 8 days after treatment. Five and 10 p.p.m. of manganese produced only yellow
chlorosis; with heavier concentrations, dark necrotic areas appeared on the leaves, later
followed by leaf distortion.

[5] The additions may have increased the pH of the soil to some extent. This, however,
probably was but a small factor in the reduction of manganese injury.

On April 17 the plants in the Berea soil averaged 15.5 inches high, and those in the Greenville soil, 11.5 inches high. The latter plants were entirely free from chlorosis. The former were still chlorotic but less so than on April 1. Photographs of these plants are shown in plate 5.

A second crop was grown in the jars without additional phosphate treatments. The duplicate jars 1 and 5 (not receiving supplementary phosphate treatments) were included. Only one plant was grown in each jar. The plants in jars 1 and 5 developed chlorosis within 5 days after planting. Later these plants became as distorted and necrotic as those in the first crop. No chlorosis developed in the plant in jar 6. A slight chlorosis developed in the plant in jar 2 some 8 days after setting. This later disappeared.

TABLE 2

Analyses of soil leachates, nitrate determinations, and pH of soils

SOIL FIELD	PLOT NO.	FIELD TREAT-MENT†	POUNDS OF THE ELEMENT PER 2,000,000 LBS. OF SOIL*						NITRATE NITROGEN		pH OF SOIL	
			Al	Ca	Cl	Fe	Mn	S	Before incubation	After incubation	Before incubation	After incubation
Berea...............	203	M	2.1	124	402	193	243	220	46.2	60.06	4.70	4.57
Berea...............	302	ML	None	300	348	Nd	22	200	76.7	112.59	6.63	6.08
Greenville..........	310	M	1.2	57	159	62	53	115	Trace	10.01	5.38	5.16
Greenville..........	307	MLSP	None	185	116	115	8	121	13.86	30.03	6.62	7.15
Mayfield............	507	M	None	70	99	68	21	68	6.84	21.00	5.25	4.94
Mayfield............	202	ML	None	350	113	194	9	194	15.40	112.59	7.02	6.84
Mayfield............	204	MSP	None	45	94	27	16	64	17.34	25.74	5.00	4.94
Campbellsville.......	713	M	None	62	119	34	14	37	13.26	37.53	5.42	5.49
Campbellsville.......	203	ML	None	213	196	47	11	60	153.90	225.00	6.24	6.48

* No water-soluble phosphate in any of the soils.
† M = manure; L = limestone; SP = superphosphate.
Nd = not determined.

In the third water culture experiment a jar was included to which 15 p.p.m. of manganese was added but the phosphorus omitted. Severe chlorosis developed in the plants in this culture. Only slight chlorosis developed on the tips of the leaves of the plants grown in the culture containing 15 p.p.m. of manganese and the usual phosphorus content. (See plate 4, fig. 2.)

Analyses of Soil Leachates

Portions of the experiment field soils weighing 2,500 gm. were incubated in the greenhouse for 2 weeks at temperatures varying from 65 to 80°F. The water content was maintained at 25 per cent of the dry soil weight. The soils were then air-dried and 2,000-gm. portions of each lot leached with 2,000 cc. of distilled water. The first portions of the leachates were poured back until

clear. The leachates were analyzed[6] for aluminum, manganese, calcium, iron, phosphorus, chlorine, and sulfur. Nitrate and pH were determined in separate samples at the beginning and the end of the incubation period.

Results of the analyses are shown in table 2. Little or no aluminum was present in any leachates. Considerable to large amounts of manganese were present in leachates from the unlimed soils. Liming materially reduced the

TABLE 3

Dry weight and ash, manganese, calcium, and phosphorus content of the plants from the soil culture experiment

Plants set January 3, 1933 and harvested April 17, 1933

SOIL AND PLOT NO.	JAR NO.	FIELD TREAT-MENT	OVEN-DRY WEIGHT OF PLANTS			PER CENT OF DRY PLANT			
			Tops	Roots	Total	Ash	Mn	Ca	P
			gm.	*gm.*	*gm.*				
Berea, 203	1	M	0.78	0.08	0.86	21.16	0.452	1.8	0.134
	2	M†	7.00	1.70	8.70	19.60	0.344	0.68	0.223
Berea, 302	3	ML	21.20	3.20	24.40	12.11	0.018	2.20	0.049
	4	ML	21.30	3.50	24.80	11.85	0.019	2.60	0.050
Greenville, 310	5	M	0.44	0.07	0.51	24.79	0.516	2.50	0.126
	6	M†	3.40	1.00	4.4	13.53	0.190	1.08	0.367
Greenville, 307	8*	MLSP	9.20	1.70	10.9	11.89	0.0023	3.00	0.064
Mayfield, 307 and 507	9	M	6.10	1.20	7.3	8.80	0.0511	1.55	0.106
	10	M	5.80	1.40	7.2	9.49	0.044	1.43	0.096
Mayfield, 204	13	MSP	8.80	1.70	10.5	9.87	0.0298	1.53	0.1231
	14	MSP	9.20	2.00	11.2	9.65	0.0334	1.43	0.1154
Campbellsville, 713	15	M	3.70	1.00	4.7	14.33	0.0790	2.32	0.0645
	16	M	3.00	0.70	3.7	15.80	0.081	2.43	0.0720
Campbellsville, 203	17	ML	8.30	2.00	10.3	14.86	0.0089	2.40	0.0934

* Jars 7, 11, 12, and 18 are not included in the table of analyses, since they were kept for further studies of the frenching which had developed in them.

† Given supplementary phosphate treatments of two 1-gm. applications of $Na_3PO_4 \cdot 12H_2O$.

soluble manganese content of these soils. Better correlation of the abnormal plant growth condition with soluble manganese than with H-ion concentration

[6] The methods used here and in analyzing the plant materials were: Aluminum, "aluminon" method as described by Winter, Thrum, and Bird (15) and as modified by Pierre, Pohlman, Gordon, and McElvane (8); manganese, periodate method as described by Willard and Greathouse (14); phosphorus, Truog colorimetric method (13); nitrate, phenoldisulfonic acid method; pH, hydrogen electrode method; calcium, McCrudden method (5); iron, chlorine, and sulfur, Methods of Analysis, A. O. A. C. (second edition revised to July 1, 1924).

is shown by the Greenville and Mayfield check soils. Plant injury was considerably greater in the former soil. Soluble manganese content was likewise considerably greater in this soil, but H-ion concentration was somewhat less. The effect of superphosphate treatments on manganese solubility is shown by comparing the Mayfield check and phosphate soils. Soluble manganese was less in the latter.

Analyses of plants

At the close of the soil experiments the plants were harvested and the roots were removed from the soil and washed clean with distilled water. After being

TABLE 4

Dry weight and manganese content of the plants from the second water culture experiment

Plants set March 18, 1933; manganese treatments added April 15, 1933; experiment ended April 28, 1933.

CULTURE SOLUTION			OVEN-DRY WEIGHT OF PLANTS			Mn IN DRY PLANT †
No. Culture	Mn	pH	Tops	Roots	Total	
	p.p.m.		*gm.*	*gm.*	*gm.*	*per cent*
1	15	4.5	2.35	0.52	2.87	0.225
2	20	4.5	1.50	0.30	1.80	0.277
3	25	4.5	1.70	0.40	2.10	0.300
4	30	4.5	1.40	0.40	1.80	0.418
5	35	4.5	2.00	0.40	2.40	0.417
6	40	4.5	2.00	0.40	2.40	0.620
7	45	4.5	2.40	0.70	3.10	0.410
8	50	4.5	1.90	0.40	2.30	0.720
9	55	4.5	1.30	0.25	1.55	1.130
10*	0	6.2	1.70	0.40	2.10	0.000
11*	0	4.5	2.15	0.37	2.52	0.000

* These treatments in duplicate; all others single.

† Slight fusion took place in ashing the plants in cultures 3, 5, 7, 9. Apparently this resulted in some loss of manganese.

oven-dried, the plants were weighed and analyzed for manganese, calcium, and phosphorus. The results are given in table 3. There is a general correlation between extent of plant injury as previously described, percentage of manganese in the plants, and amount of manganese in the soil leachates (table 2).

The question arises as to whether the effect of the lime and phosphate treatments in preventing or decreasing plant injury was entirely due to their action in lowering the solubility of manganese in the soil. No other explanation seems necessary in the case of the lime treatments, but this apparently is not true for the phosphate treatments. Perhaps the addition of phosphate had an additional effect in making manganese inactive within the plant. The high percentage of manganese in the plants grown in the Berea and Greenville check jars which received supplementary phosphate treatments (jars 2 and 6, table 3)

is evidence in this direction.   Pierre and Stewart (9) have lately suggested that the effect of phosphate treatments in reducing aluminum injury is in part caused by precipitation of aluminum within the plant.

Results of analyses of plants in the second water culture experiment are given in table 4.   The percentage of manganese in the plants increased with increase in manganese in the culture solutions.

TABLE 5

Dry weight and manganese and phosphorus content of the plants from the third water culture experiment

Plants set and treated on April 15, 1933; experiment ended April 28, 1933

CULTURE SOLUTION			OVEN-DRY WEIGHT OF PLANTS			PER CENT OF DRY PLANT	
No. Culture	Mn	pH	Tops	Roots	Total	Mn	P
	p.p.m.		*gm.*	*gm.*	*gm.*		
4	15*	4.5	0.50	0.15	0.65	0.923	0.194
5	15	4.5	0.50	0.20	0.70	0.460	0.700
6	20	4.5	0.59	0.15	0.74	0.371	0.690
7	30	4.5	0.48	1.14	0.62	0.443	0.680

* This culture received no phosphorus.

TABLE 6

*Per cent of manganese and phosphorus in the plants of the second crop of Turkish tobacco grown in the Berea and Greenville soils**

JAR NO.	SOIL AND TREATMENTS	Mn			P		
		Water-soluble	Insoluble in water	Total	Water-soluble	Insoluble in water	Total
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	Berea M	0.1100	0.0460	0.1560	None	0.0014	0.0014
2	Berea M plus P†	0.0110	0.0009	0.0119	0.0033	0.0073	0.0106
3	Berea ML	None	Trace	Trace	0.0072	0.0115	0.0187
5	Greenville M	0.0730	0.0203	0.0933	None	0.0049	0.0049
6	Greenville M plus P†	0.0027	0.0068	0.0095	0.0043	0.0082	0.0125

* All percentages calculated on the green weight of plant.
† Phosphorus treatments in jars 2 and 6 added as a supplementary treatment for the first crop.

Results of analyses of plants in the third water culture experiment are given in table 5.   There was a large increase in percentage of manganese in the plants grown in the culture where phosphorus was omitted as compared with the corresponding culture containing phosphorus.   Increase in manganese concentration in the culture solutions also failed to bring about increase in percentage of manganese in the plants.   In both these respects the results are not in line with those previously given.   It is to be considered that in this experiment the plants were started directly in the culture solutions containing the special treatment and grew only a relatively short time.

Kelly (4) reports work showing a considerable variation in the degree of solubility of the plant manganese in different plants. To obtain information on this point for Turkish tobacco, the plants in the second crop in the Berea and Greenville jars 1, 2, 3, 5, and 6 were harvested (50 days after the plants were set). The stem and leaves were ground with a little distilled water in a mortar until thoroughly macerated and then leached with distilled water. The leachate was analyzed for manganese. The residue was ashed and also analyzed for manganese. The results are shown in table 6. Excepting the plant from jar 6, containing only a low total percentage, most of the manganese was soluble.

SUMMARY AND CONCLUSIONS

Studies were made to determine the cause of a chlorosis in Turkish tobacco grown in soils from unlimed plots of several of the Kentucky outlying soil experiment fields. Previous work here and elsewhere suggested that it was manganese.

Plants were grown in the greenhouse in soils from check and from limed and phosphated plots from the Berea, Greenville, Mayfield, and Campbellsville soil experiment fields, and the extent of injury which developed was noted. Severe to slight injury developed in plants in all the unlimed soils. No injury developed in limed soils. Phosphate treatments very much reduced the injury.

Plants were grown in water cultures containing varying amounts of alunum and manganese. Aluminum additions injured the roots, but there was no effect similar to that in the plants grown in the soils. Manganese in concentrations of 15 p.p.m. in the culture solution used, produced chlorosis in the plants. This became more severe with higher manganese concentrations. The condition of the plants appeared to be the same as that of the plants grown in the soils. Removal of phosphorus from the culture solution lowered the manganese concentration at which injury took place. Cultures were also included to determine the effect of variations in pH. A pH as low as that of any of the check soils had no injurious effect on plant growth.

Additional portions of the soils used in the soil-plant experiments were incubated in the greenhouse for 2 weeks then treated with distilled water and the leachates analyzed for manganese, aluminum, and other constituents. Very little or nor aluminum was found in the leachates. Considerable to much manganese was found in leachates from the soils of the check plots. Liming very largely reduced manganese solubility.

Analyses were made of plants grown in the soils. There was general correlation between extent of plant injury, percentage of manganese in the plants, and amount of manganese in the leachates.

Analyses of plants grown in water cultures with manganese concentrations from 15 p.p.m. to 55 p.p.m. showed the percentage of manganese in the plants to increase from 0.225 to 1.13.

It was concluded that the abnormal growth conditions developing in Turkish tobacco grown in soils from the check plots of outlying Kentucky soil fields is due entirely to soluble manganese. There was some evidence that part of the effect of the phosphate treatment in reducing plant injury was through making manganese inactive in the plant. The findings of this experiment suggest that soluble manganese may be an important factor in the unfavorable effects on crop growth of the acid condition of many soils in Kentucky.

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PLATE 1

TOXIC CONDITION IN TURKISH TOBACCO PLANTS 10 DAYS AFTER BEING SET; CONSIDERABLE CHLOROSIS IN UNLIMED SOILS; NONE IN LIMED SOILS

FIG. 1. Berea soil: a, check soil; b, limed soil.

FIG. 2. Greenville soil: a, check soil; b, limed soil.

FIG. 3. Campbellsville soil: a, check soil; b, limed soil.

FIG. 4. Mayfield soil: a, check soil; b, superphosphated soil; c, limed soil.

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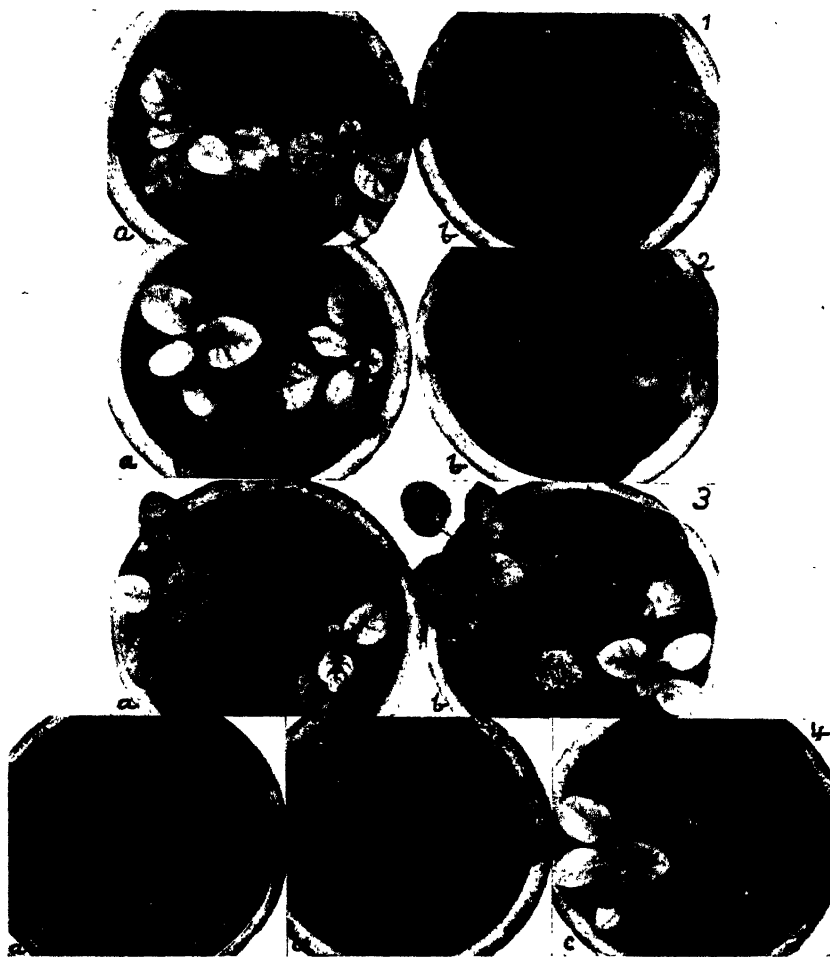
Figs. 1–4

PLATE 2

SEVERE TOXIC CONDITION IN TURKISH TOBACCO 49 DAYS AFTER BEING SET; NO TOXICITY
IN LIMED SOILS

FIG. 1. Berea soil: a, check soil; b, limed soil.

FIG. 2. Greenville soil: a, check soil; b, limed soil. Phosphorus-deficient spotting on
two leaves.
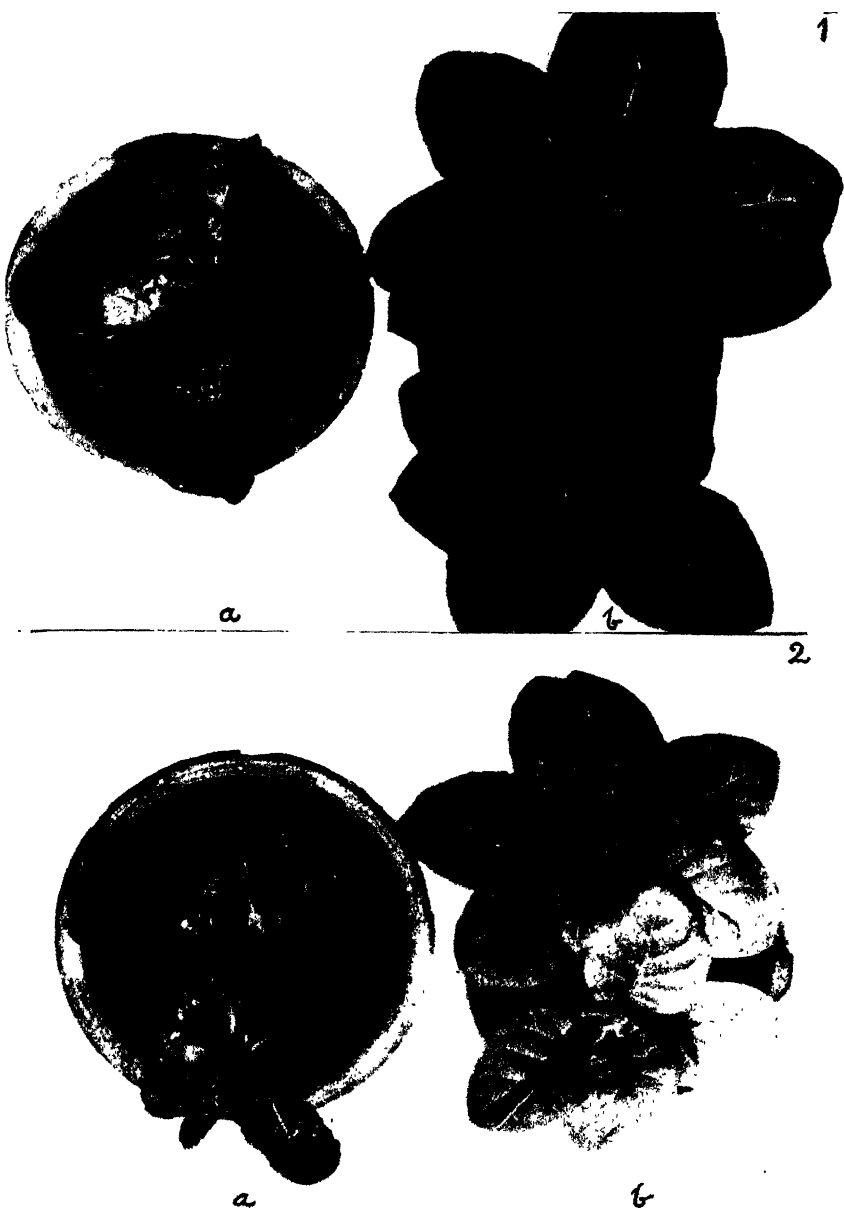
Figs. 1–2

PLATE 3

TURKISH TOBACCO PLANTS, 49 DAYS AFTER BEING SET, SHOWING COMPARATIVE SIZE, TOXIC CONDITION IN PLANTS FROM BEREA AND GREENVILLE CHECK SOILS, AND RECOVERY IN THE CAMPBELLSVILLE AND MAYFIELD UNLIMED SOILS; NO TOXICITY IN THE LIMED SOILS

FIG. 1. Berea soil: a, check soil; b, limed soil.

FIG. 2. Greenville soil: a, check soil; b, limed soil.   The lower leaves on these plants as well as those in figure 1b show spotting due to phosphorus deficiency.

FIG. 3. Campbellsville soil: a, check soil; b, limed soil.

FIG. 4. Mayfield soil: a, check soil; b, superphosphated soil; c, limed soil.

Figs. 1–4

29

PLATE 4

Toxic Condition of Turkish Tobacco in Water Cultures Containing Manganese; Photographed 13 Days After Mn Additions

Fig. 1. Plants from Experiment 2: (a) Full nutrient solution plus 40 p.p.m. of Mn. Severe chlorosis with brown necrotic areas. (b) Full nutrient solution; no Mn. No chlorosis.

Fig. 2. Plants from Experiment 3. showing effect of phosphorus in reducing manganese injury: (a) Nutrient solution containing no phosphorus, plus 15 p.p.m. of Mn. Severe chlorosis with some necrotic areas. Phosphorus deficient spot on one leaf. (b) Full nutrient solution plus 15 p.p.m. of Mn. Slight chlorosis in growing point.

Figs. 1–2

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PLATE 5

EFFECT OF SUPPLEMENTARY PHOSPHATE TREATMENTS IN OVERCOMING THE TOXIC CONDITION
IN TURKISH TOBACCO GROWN IN BEREA AND GREENVILLE CHECK SOILS; PHOTOGRAPHED
48 DAYS AFTER THE FIRST TREATMENT WAS ADDED

FIG. 1. Berea soil: (a) Two 1-gm. additions of $Na_3PO_4 \cdot 12H_2O$. Some chlorosis in growing point. (b) No phosphorus additions. Severe toxic condition.

FIG. 2. Greenville soil: (a) Two 1-gm. additions of $Na_3PO_4 \cdot 12H_2O$. No chlorosis. (b) No phosphorus additions. Severe toxic condition.

Figs. 1-2

MINERAL CONSTITUENTS IN RELATION TO CHLOROSIS OF ORANGE LEAVES

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Chlorosis or yellowing of orange leaves has been observed in many of the orange groves in the Gosford district, N. S. Wales, for several years, and is gradually becoming more prevalent. The affected leaves first show diffused yellow interveinal areas, not specially demarcated from the green tissue and rather toward the petiole end of the leaf. Gradually the whole leaf assumes a yellow color, with a greenish tinge along the main veins and midrib, but these eventually become yellowish. The chlorotic leaves tend to occur more on the northern or northwestern aspects of the trees, those aspects receiving the greatest intensity of light. The affected leaves are narrower than normal leaves, and in moderately affected trees sometimes occur in patches confined to individual limbs. In badly affected trees, yellow leaves are distributed over the whole surface. No bunching of small twigs accompanies the disease, but a marked abscission of yellow leaves takes place during the winter months.

Chlorosis and mottling of orange leaves have been reported from many parts of the world, though usually ascribed to conditions other than those that obtain at Gosford.

Chlorosis is a diseased condition of plant leaves induced by some abnormality in the supply of nutrients, which results in a failure to produce a normal amount of chlorophyll, and thereby the amount of material synthesized by affected leaves is reduced. Compounds of nitrogen, iron, and magnesium are intimately associated with the production of chlorophyll (17), and frequently when soil conditions are such as to render the uptake of these substances insufficient for plant requirements a chlorotic condition is produced.

Hilgard (8) reports a yellowing of the leaves and general unthriftiness of citrus trees grown on Californian soils which have an accumulation of calcium carbonate in the subsoil. The alkalinity of these soils evidently results in the immobilization of iron compounds, and a chlorotic condition resulting from a lack of iron ensues. Many methods have been used to overcome a lack of iron in citrus (2, 15). Thomas and Haas (14) found that soil applications of sulfate of iron up to 100 pounds per tree had no effect. When aged Valencia trees were injected with 2 liters of water containing 10 gm. of iron tartrate, a marked abscission of the yellow leaves resulted, followed by new growth from the axillary buds. The new growth was of normal color, and fruit production

increased, but in less than 2 years the leaves of the succeeding cycles of growth were of pale color, indicating the temporary nature of the improvement.

Kelley and Thomas (11) describe the effect of alkali on orange trees—on some soils mottle-leaf is one of the early symptoms, whereas trees on soils containing specially high concentrations of sulfates and bicarbonates exhibit a chlorotic condition similar to that referred to by Hilgard on calcareous soils.

COLLECTION OF SAMPLES

Some difficulty was experienced in locating trees which were totally free of evidences of chlorosis, for such trees form a very decided minority in the various groves visited. Samples of leaves were collected on November 24, 1931, at a time when there was little new growth on the trees. Since care was taken to avoid young leaves, the material collected represents mature leaves ranging in age from probably several months to 2 years or over. From 200 to 300 leaves were collected from a tree, and each sample represents leaves from a single tree. There are very notable differences in the composition of immature and mature orange leaves. Young leaves contain very much higher percentages of phosphorus, iron, potash, and nitrogen than do old leaves (10), and there is a gradual diminution of these constituents as growth proceeds. The calcium content is exceptional in that it is low in young leaves and increases in amount until full maturity is reached.

The leaves were washed individually, air dried, and then ground in a hand mill and stored in bottles. The petioles were not included in the prepared samples. In table 1 a description of the samples is given.

Soil samples were collected from holes dug within several feet of the trees, and are given numbers corresponding to the number given the leaf samples of the respective trees. Soils in the Gosford district range from dark-gray to yellowish loamy sands or sandy loams, passing into the B horizon at 18 to 20 inches. The latter consists of yellowish-brown or orange loams, or clayey sands, which overlie sandstone, or ferruginous indurated sandstone.

The samples consist of A1 and A2 horizons to a depth of 18 to 20 inches. The largest portion of the trees' roots is in this zone, for there is a decided tendency for the longer roots to run above and parallel to the B horizon.

THE SOILS AT GOSFORD

A general reference to the soils at Gosford with respect to profile characteristics has already been made. One can well deduce the type of soils from a consideration of the climatic conditions. Moderate temperatures combined with a 45-inch rainfall have resulted in the light-textured soils derived from sandstone being very much leached and strongly acid in reaction; pH values of 4.2 are frequently encountered. The topography is steep to rugged with valley alluvium, and where soil has been sufficiently long in situ, it has devel-

oped strongly podzolic characters, but considerable areas of the soils are of the nature of truncated podzols and consist largely of soils representing B hori-

TABLE 1

Description of the leaf samples

GROVE	SAMPLE NUMBER	VARIETY	NATURE OF LEAF SAMPLES
W. Holcombe	1*	Valencia	Green leaves from healthy tree
	2	Valencia	Green leaves from healthy tree
	3	Valencia	Yellow leaves from affected tree
	4	Valencia	Yellow leaves from affected tree
	5	Valencia	Green leaves from healthy tree
	6	Valencia	Green leaves from healthy tree
E. A. Bailey	7	Washington Navel	Green leaves from badly affected tree
	7A	Washington Navel	Yellow leaves from same tree as 7
	8	Washington Navel	Green leaves from badly affected tree
	8A	Washington Navel	Yellow leaves from same tree as 8
	9 10	Washington Navel	Green leaves from adjacent trees, showing slight evidences of yellowing
	11	Valencia	Yellow leaves from badly affected tree
	12	Valencia	Green leaves from healthy tree
H. Westcott	13	Valencia	Yellow leaves from badly affected tree
	14	Valencia	Yellow leaves from badly affected tree
	15	Washington Navel	Yellow leaves from affected tree
	16	Washington Navel	Green leaves from tree showing trace of yellowing
N. Scholer	17	Valencia	Yellow leaves from badly affected tree
	18	Valencia	Yellow leaves from badly affected tree
N. E. Pinkston	19	Valencia	Green leaves from tree showing trace of yellowing
	20 21	Valencia	Green leaves from younger healthy trees 7 to 8 years old
	La LB	Valencia	Green leaves from healthy trees at Leeton, Murrumbidgee Irrigation Area, N.S.W.

* Trees 1–6 are located in a fertilizer experiment (3) and have annually since 1925 received the following treatment per tree:
1 and 2. 5 pounds bone dust, 5 pounds superphosphate, 4 pounds sulfate of potash, 9 pounds sulfate of ammonia.
3 and 4. The same as 1 and 2, but with only 4 pounds sulfate of ammonia.
5 and 6. The same as 1 and 2, but with potash omitted.

zons of former soils. Table 4 gives the reaction, nitrogen, and replaceable base content of soils numbered to correspond with trees from which leaf samples were taken.

DISCUSSION

In comparing the chemical composition of chlorotic leaves with normal leaves, many similar features present themselves in widely varying plant families. Wallace and Mann (16), in their investigations of lime-induced chlorosis of apple trees, found that there was a higher ash, potassium, and sodium (especially potassium) content in the chlorotic leaves; a much higher content of calcium, together with a somewhat greater magnesium content in the green leaves, whereas the amounts of iron, aluminum, phosphorus, and silica were not greatly different. The author in unpublished data has compared the composition of normal and chlorotic peach leaves of the same variety, the diseased trees being located on an area at Leeton, Murrumbidgee Irrigation Area, on which a previous citrus crop had developed chlorosis. There was a higher ash, potassium, sodium, manganese, phosphorus, and silica content in the yellow leaves, and higher lime and nitrogen content in the green.

Haas, Batchelor, and Thomas (6) report that "yellows" or "little-leaf" of walnut trees, rosette of pecan trees, as well as little-leaf of peach, and mottling of apricot have been found to be independent of nitrogen requirements, cultural practices, and nematode infection, but appear to be dependent on the base relationship existing in the soil. The ash of the affected leaves is lower in calcium and higher in potassium than that of healthy leaves of the same age.

Comparing the composition of normal and mottled orange leaves, Kelley and Cummins (10) found that mottled leaves contained greater amounts of potassium, phosphorus, magnesium, sulfate, and nitrogen. Normal leaves contained a greater amount of calcium, whereas the iron, silica, and chlorine contents did not materially differ.

Neither in the symptoms of mottle leaf as it occurs in California (4) and in chlorotic leaves at Gosford nor in the relation of the diseases to normal leaves in the same locality are there many parallel features. In the former, irregular spots several millimeters in diameter appear between the larger veins and may become larger and more numerous until the only chlorophyll remaining is confined to the midrib and main veins. A specific feature is that the areas surrounding the yellow spots retain their normal green color, at least till the spots embrace a large proportion of the leaf. In the Gosford samples the percentage of potassium is significantly higher in the chlorotic leaves than in the green leaves of normal trees. Of the latter 9 and 10 are exceptional in having a relatively high potassium content but have as yet not developed any considerable degree of chlorosis. They are, however, situated near trees which show much evidence of yellowing, and the disease will probably soon become more marked. It appears that a high potassium content may precede a serious manifestation of the disease. Of the chlorotic leaves 13 and 14 are exceptional in that they do not have a high potassium content. It is, therefore, a usual but not a necessary accompaniment of chlorosis that leaves have a high potassium content. A low potassium content associated with yellowing may

TABLE 2

Composition of green leaves from healthy or slightly affected trees

Ash, constituents of ash, and nitrogen, expressed as percentage of dry matter

NUMBER	VARIETY	ASH	SiO₃	CaO	MgO	K₂O	Na₂O	MnO	Fe₂O₃	SO₄	Cl	P₂O₅	N
1	Valencia	13.23	.413	6.36	.314	0.772	.156	.0018	.066	.371	.121	.236	2.26
2	Valencia	13.16	.328	6.31	.403	0.706	.191	.0020	.043	.414	.113	.227	2.26
5	Valencia	14.30	.415	7.10	.471	0.495	.253	.0016	.039	.475	.112	.229	2.22
6	Valencia	13.92	.350	6.73	.536	0.445	.238	.0018	.033	.418	.115	.241	2.28
12	Valencia	12.56	.352	5.66	.860	0.562	.196	.0011	.055	.451	.103	.198	2.17
19	Valencia	10.30	.171	4.84	.186	0.788	.228	.0007	.035	.409	.103	.213	2.21
20	Valencia	14.81	.191	7.08	.379	0.992	.385	.0014	.030	.398	.123	.230	2.15
21	Valencia	14.48	.196	7.12	.330	0.925	.349	.0011	.033	.370	.123	.229	2.16
9	Washington Navel	12.38	.660	4.84	.351	1.492	.307	.0015	.061	.581	.168	.264	2.38
10	Washington Navel	11.74	.660	5.06	.286	1.425	.304	.0020	.056	.619	.128	.233	2.19
16	Washington Navel	12.02	.375	5.97	.386	0.768	.324	.0022	.036	.582	.172	.232	2.57
LA*	Valencia	13.55	.721	5.40	.780	0.721	.112	.0023	.081	.772	.021	.259	2.26
LB*	Valencia	12.17	.678	5.14	.627	0.830	.197	.0019	.126	.667	.027	.241	2.43
Mean...		13.05	.366	6.11	.410	0.864	.264	.0015	.044	.462	.125	.230	.227
		±.251	±.058	±.164	±.031	±.181	±.012	±.000025	±.0024	±.017	±.0046	±.0034	±.0023

* Not included in mean.

TABLE 3

Composition of chlorotic and green leaves from trees showing chlorosis

Ash, constituents of ash, and nitrogen, expressed as percentage of dry matter

NUMBER	VARIETY	ASH	SiO₄	CaO	MgO	K₂O	Na₂O	MnO	Fe₂O₃	SO₄	Cl	P₂O₅	N
3	Valencia	13.60	.666	5.93	.103	1.383	.334	.0030	.048	.398	.186	.237	1.94
4	Valencia	13.28	.638	6.13	.114	1.176	.395	.0013	.042	.425	.248	.238	2.04
11	Valencia	13.70	.453	6.64	.052	0.943	.299	.0012	.045	.462	.202	.229	2.15
13	Valencia	11.69	.437	5.52	.062	0.725	.263	.0028	.043	.546	.187	.213	2.28
14	Valencia	12.72	.578	6.41	.052	0.613	.317	.0033	.040	.624	.232	.210	2.13
17	Valencia	12.62	.385	6.14	.052	1.043	.395	.0006	.033	.786	.132	.285	1.86
18	Valencia	13.17	.358	6.36	.044	1.051	.333	.0007	.037	.404	.152	.245	1.77
7	Washington Navel	13.32	.395	5.85	.213	1.603	.189	.0014	.049	.423	.180	.249	2.38
7A	Washington Navel	14.84	.604	6.12	.079	2.223	.386	.0022	.040	.485	.186	.231	2.07
8	Washington Navel	13.25	.438	5.40	.151	1.728	.249	.0015	.060	.548	.126	.256	2.38
8A	Washington Navel	13.44	.515	4.93	.099	2.475	.416	.0015	.055	.437	.153	.282	2.22
15	Washington Navel	11.74	.536	5.70	.047	0.943	.341	.0018	.052	.406	.213	.159	2.21
Mean		13.08	.508	5.93	.096	1.300	.327	.0018	.045	.495	.178	.235	2.12
		±.156	±.021	±.093	±.0108	±.109	±.013	±.00015	±.0014	±.023	±.0069	±.0065	±.0054

ensue from a limited soil potassium supply, but the latter condition in relation to the disease does not appear to be causal.

Bartholomew and Janssen (1) have shown that plants will absorb large amounts of potassium, apparently in excess of that required for normal growth, when excessive amounts are made available. Under these conditions potassium does not enter into organic combinations but remains in a water-soluble condition.

The same authors (9) working with tomatoes in nutrient solution found that nitrogen and potassium bear an inverse relationship in that wherever a high percentage of potassium is found in the plants a low percentage of nitrogen is likely to occur.

Though there is in general a higher potassium and a lower nitrogen content in the yellow leaves, individual considerations do not support the theory that the high potassium content of the yellow leaves may be due wholly to an insufficient nitrogen supply.

In considering the composition of leaves 1–6, some effects apparently due to the fertilizer treatment can be deduced. Trees 1 and 2 have received potassic fertilizer and relatively high amounts of nitrogen. Trees 3 and 4 have received the same amount of potassium but less than half the amount of nitrogen; there has been a considerably greater uptake of potassium by the yellow leaves together with a much diminished amount of magnesium and somewhat less nitrogen. Trees 5 and 6 have received no potassium but the same amount of nitrogen as 1 and 2; the leaves are normal, the potassium content is low but the magnesium content is high and no disease symptoms have attended the with-holding of potassium supplies. Reed and Haas (13) in experiments with orange trees grown in nutrient solutions found that orange trees can function with considerably less potassium than they ordinarily absorb, and young trees grow fairly well over an experimental period of 17 months in a solution devoid of potassium, being able to subsist on the potassium contained in their tissues at the outset of the experiment. The effect of fertilizer treatment on 3 and 4 does suggest that a low nitrogen supply has contributed to disease conditions and high potassium content, and especially to a feature common to all the samples of chlorotic leaves, namely, a low content of magnesium. The magnesium deficiency of chlorotic leaves is the most striking feature in a comparison of their composition with normal leaves.

The mean content of magnesium in the normal leaves is 0.41 ± 0.0313 per cent and that of chlorotic leaves 0.096 ± 0.0108. No. 19 represents the normal foliage of a tree in the same grove as 20 and 21, though the latter are younger trees, and on soil that has been under cultivation for a shorter period. The magnesium content has fallen to a lower figure in the older tree, whereas in the younger trees the magnesium content is fairly high. Coincident with the low magnesium content of the older tree is the early appearance of yellow leaf; the effects of continued cropping and soil impoverishment are reflected in the low total mineral content.

A good deal of prominence has of late years been given to the rôle of magnesium as a limiting factor in plant nutrition, and specific diseases have been attributed to the lack of this constituent.   Chucka (5) reports that a chlorotic appearance of potatoes, known as "potato sickness," could be prevented by the addition of magnesium to potato fertilizers.   McMurtry (12) reports that when magnesium is deficient in tobacco soils, the deficiency gives rise to a chlorosis of tobacco leaves, which is usually worse on sandy soils.   The specific symptoms of magnesium deficiency in regard to tobacco is the loss of the green color at the tip and margins of the lower leaves of the plant—in some cases the lower leaves of the plant may be almost white.

In 7 and 7A and 8 and 8A a comparison is made of the composition of green and chlorotic leaves derived from trees showing a diseased condition.   The green leaves show the same general features of the mineral content of chlorotic leaves especially in an abnormal potassium content, but the abnormalities are greatly accentuated in the yellow leaves.   The magnesium content of the green leaves is low but in the yellow leaves is considerably less.   There are no significant differences in the ash, calcium, iron, sulfate, or phosphate contents of the normal and diseased leaves.

There is a higher content of silica, sodium, and chlorine in the yellow leaves. The manganese content of the latter is also higher but in a barely significant degree.   There is a slightly higher nitrogen content in the normal leaves.

LA and LB are two samples of healthy Valencia orange leaves collected from two trees in a grove at Leeton, Murrumbidgee Irrigation Area, N. S. Wales.   They were included in the analyses for comparison with leaves from healthy trees at Gosford.   These two districts are very dissimilar in climatic, and consequently soil, conditions.   Despite the more basic nature of the soils at Leeton, the composition of the leaves is similar to those at Gosford, viz., comparatively low potassium and sodium, high magnesium, similar nitrogen and phosphorus, and a very low chlorine content.

Some interest attaches to the high chlorine content of chlorotic leaves, and a tendency to a higher sulfate content than leaves of normal trees.   Haas and Thomas (7) report injuries to the leaves of lemons as a result of the accumulation of sulfur compounds, and that the use of generous additions of nitrogenous fertilizers somewhat decreases the toxic effects due to sulfate and chloride concentration in the leaves by increasing the leaf area.

An examination has been made of the replaceable magnesium, calcium, and potassium, of the soils corresponding to the leaf samples, to determine whether there is any correlation between the amount of replaceable bases and the amount of bases occurring in the leaves of the trees, especially where a deficiency occurs in the leaves.

The replaceable calcium content of the soils is low throughout, and very low in many samples.   It corresponds with amounts of this base found in light-textured acid soils in other parts of the world, or in light-textured acid soils from which calcium has been largely lost by the use of ammonium salts as a

fertilizer.   Since ammonium sulfate is a cheap and effective source of nitrogen in citrus fertilizer practice, the already low calcium content will become aggravated.   Citrus trees contain notably large amounts of calcium in comparison with other crop plants, and there is a distinct possibility of a calcium hunger being induced on these soils.   Oranges are grown in many parts of the world under semi-arid conditions, and in soils rich in bases.   In considering the vastly different conditions at Gosford—leached, acid, base-poor soils— it is evident that much stronger influences than the amount of nutrients available to a plant govern its composition, and the most potent factor would appear to be heredity.

TABLE 4

Reaction, nitrogen, and replaceable base content of soils

SOIL NUMBER	NITROGEN	pH	REPLACEABLE BASES EXPRESSED AS M.E. PER 100 GM.		
			CaO	MgO	K₂O
	per cent				
1	.105	5.3	3.38	.505	.343
3	.092	5.0	2.67	.359	.242
4	.085	4.8	2.18	.288	.226
5	.104	4.8	2.48	.288	.191
7	.085	4.2	1.08	.324	.189
8	.088	4.2	1.69	.281	.275
9	.101	4.6	1.72	.327	.416
11	.137	4.6	2.10	.395	.327
12	.143	5.2	3.57	.891	.245
13	.083	4.4	1.38	.308	.159
15	.110	4.9	1.30	.184	.104
16	.120	4.4	1.92	.381	.127
17	.051	5.0	1.24	.266	.085
19	.046	5.3	1.50	.137	.077
20	.053	5.3	1.81	.252	.119

Though in these soils the amount of bases is such that almost no single one can be very remote from being a limiting factor, there is no significant degree of correlation between the available bases of the soils, as measured by their replaceability, and the amounts of those bases occurring in the leaves.

The magnesium content is also low and typical of leached acid soils.   The soils containing most magnesium are associated with trees whose leaves have a high magnesium content, as those containing least are associated with chlorotic trees, but considering the whole series there is no significant correlation.

A few of the soils appear moderately well supplied with potassium, but the majority have small reserves of this base.   Iron appears to be in a sufficiently mobile condition in these soils, for an appreciable amount is extracted by a normal solution of ammonium chloride.

SUMMARY

Chlorosis of orange trees is becoming an increasingly serious disease at Gosford, N. S. Wales. The symptoms of the disease are described.

Analyses of normal leaves from healthy or very slightly affected trees, and of chlorotic leaves are given.

The salient feature in comparing the mineral composition of healthy and diseased leaves is the deficiency of magnesium in the latter.

The normal leaves have a significantly greater content of magnesium and nitrogen.

The content of potassium, sodium, silica, and chlorine is higher in the chlorotic leaves. Ash, calcium, manganese, iron, sulfate, and phosphate contents are not significantly different.

The composition of normal orange leaves grown under vastly different soil and climatic conditions differs little from normal leaves grown at Gosford.

Soil conditions at Gosford are briefly discussed, and the major replaceable base constituents of a number of soils are given.

There is no significant degree of correlation between replaceable bases in these soils, and the uptake of the corresponding bases.

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CARBON DIOXIDE PRODUCTION BY MANNITE-TREATED SOILS AS A MEANS OF DETERMINING CROP RESPONSE TO FERTILIZERS

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Since running field fertilizer tests on all soil types and on all parts of the same soil which have different fertilizer needs is impracticable, investigators have developed rapid laboratory methods for determining soil deficiencies. Certain investigators have used the rate of evolution of carbon dioxide as a measure of the activity of the soil microörganisms. This method has the advantage over some biological methods in that the determinations are quantitative. Soil microörganisms not only require an energy-giving material, but they also require approximately the same nutrients which crop plants require. The activity of the soil microörganisms in the presence of an energy material, and therefore the rate of evolution of carbon dioxide, should be a function of the nutrients available for crops, other growth factors being taken care of.

Waksman and Starkey (5) found that soils could be graded on a basis of their fertility by determining the amount of carbon dioxide formed by 1 kgm. of fresh soil in 14 days or by determining the amount of carbon dioxide produced in 48 hours by 100 gm. of fresh soil to which 500 mgm. of dextrose had been added, as well as by determining the numbers of microörganisms and the nitrification.

Waksman and Heukelekian (3) found that when cellulose was added to a soil 40 to 50 mgm. of cellulose was decomposed in 14 days for every milligram of available nitrogen present. They measured the decomposition of the cellulose by determining the carbon dioxide evolved.

Corbet (2) and numerous others have determined the effect of the addition of various fertilizing salts on the rate of evolution of carbon dioxide by a soil. Usually they did not add an energy material to the soil; consequently, the increases in carbon dioxide obtained were either negligible or small.

Waksman and Karunakar (4) found that information concerning the micro-biological flora capable of fixing nitrogen and concerning the available phosphorus in a soil could be obtained by the following methods:

1. "The common solution method, consisting in adding 1 or 5 gm. of soil to a standard mannite solution, incubating for 7 to 28 days, then determining the increase in total nitrogen above the control. This serves as an index of the nitrogen fixing flora of the soil and to some extent of the microbiological condition of the soil.

2. "The method suggested by Niklewski (—) and Stoklasa (—). This consists in adding 10 gm. of the particular soil to 100 cc. of 2 per cent mannite solution, free from available phosphates, sterilizing and inoculating with a vigorous culture of azotobacter. After incubating for 20 to 30 days, the increase in total nitrogen is determined. This can serve as an index of the available phosphate in the soil.

3. "The method for determining residual mannite (or rather soluble organic matter) in the soil suggested by Christensen (—). This consists in adding 2 per cent of mannite to the soil, incubating with optimum moisture, then determining the residual mannite every five days by oxidation with $KMnO_4$. This method as well can serve as an index of the activities of the nitrogen fixing flora and of the amount of phosphorus available in the soil."

Waksman and Karunakar concluded that nitrogen fixation on mannite treated soil is an unreliable index of microbiological activity because the quantities of nitrogen are small and the methods for determining nitrogen are not sufficiently sensitive to measure them. They found much more applicable the method of Christensen in which mannite disappearance is measured.

In the South, nitrogen and phosphorus are the nutrients must often deficient in the cultivated soils. All microbiological methods in soil investigation are based upon the fact that crop plants and soil microörganisms require similar nutrients for their development: the plants obtain their energy from the sun, and the lower organisms obtain theirs from energy-giving compounds.

The main differences in the method suggested by Christensen and the method proposed in this paper are: (a) Christensen used water on the soil equal to 75 per cent of the maximum water-holding capacity of the soil, whereas in the method proposed in this paper $33\frac{1}{3}$ per cent of the maximum water-holding capacity is used; (b) Christensen determined the residual mannite, whereas in the method of this paper the carbon dioxide, an end product of respiration, is determined; (c) the determinations required by Christensen's method are one every 5 days until 30 days have passed, whereas only one determination and 1 day are required by the proposed method.

The water used by Christensen, which is much greater than that which the plants under usual cultivation conditions are subjected to for any length of time, is sufficient to influence markedly the solubility of the soil phosphates, and it is reasonable to assume that maintenance at this moisture content for some time will alter the absorption of phosphorus from the soil by the microörganisms.

The moisture used in the proposed method is $33\frac{1}{3}$ per cent of the maximum water-holding capacity, and the soil is friable and loose as in a freshly cultivated field. The conditions as to moisture and air are similar to those of a freshly cultivated field.

When mannite or any readily available energy material is added to the soil the conditions are made as favorable for the soil microörganisms as for crop plants. If sufficient nutrients are available, both the production of CO_2 by the

soil microörganisms and the crop yield will be large. If nitrogen or phosphorus is deficient in a soil the addition of the deficient element to a field will increase the crop yield, likewise its addition to a mannite-treated soil will increase the production of CO_2 by the soil microörganisms.

The time (24 hours) required in the proposed method is sufficiently long for responses to nitrogen and phosphorus to show up and sufficiently short to prevent the nitrogen fixed by the soil flora from markedly affecting the production of CO_2.

Waksman and Starkey (5), Waksman and Heukelekian (3), Christensen and Jensen (1), and Corbet (2) have reviewed the literature relative to carbon dioxide production and microbiological analysis of the soil.

The object of the investigation reported here was to determine the relationship between the activity of the soil microörganisms in the soil to which mannite had been added, as measured by CO_2 production, and the fertilizer requirement of the soil for cotton production as measured by crop yields.

For the laboratory work in this investigation soil samples were collected from 13 different experimental fields in Mississippi and Louisiana on which fertilizer tests for cotton had been, or were being, conducted and for which crop yields are available. The samples of soil were taken from the unfertilized plots where the experiment was still in progress and at random over the field where the experiment had been discontinued. In the laboratory after preliminary tests the following ingredients were added to duplicate 100-gm. samples of air-dry soil:

1. 500 mgm. mannite.
2. 500 mgm. mannite and 30 mgm. of nitrate of soda for the Mississippi soils, or 25 mgm. for the Louisiana soils.
3. 500 mgm. mannite, nitrate of soda,[2] and 25 mgm. superphosphate.

After the soil and the added ingredients had been thoroughly mixed, water equal to 33⅓ per cent of the maximum water-holding capacity of the soil was added. The maximum water-holding capacity was taken as that amount of water which is retained in a soil in a filter funnel when water is added in excess, and the excess allowed to drain off. The soil so treated was then pulverized, care being taken to distribute the moisture uniformly, after which it was put into 1,000-cc. Erlenmeyer flasks. The flask was closed with a 2-hole rubber stopper carrying a long glass tube extending to the bottom of the flask and a short glass tube extending just through the stopper. The flask was made airtight by putting over the outer ends of the tubes rubber tubes closed at one end with pointed glass stoppers. Glass wool was put over the inside end of the short glass tube to exclude the soil. Then the flask with its contents was inverted into its support and incubated for 24 hours at room temperature in summer and at 30°C. in winter. After the incubation period of 24 hours the flask was connected in a gas train for the collection of the carbon dioxide. The members of the train in order were: soda lime column, soil flask, H_2SO_4 tube, $CaCl_2$ tube (improvised), ascarite tube (improvised from a 1-inch by 6-inch

[2] See number 2 for the quantity.

test tube, a 2-hole rubber stopper, and glass tubing), $CaCl_2$ tube, and a 3,600-cc. water bottle from which water was siphoned in order to draw air through the train. The soil flask was so connected that the air entered it through the long tube and passed downward through the soil and out through the short tube thus facilitating the removal of the carbon dioxide.

The carbon dioxide was swept out of the soil flask by drawing 3,600 cc. of CO_2-free air through the train. The CO_2 was absorbed by the ascarite and determined by weighing the ascarite tube before and after the absorption. The ascarite tubes were stored in $CaCl_2$ desiccators when not in use. The change in weight of the tube between one determination and the next was not sufficient to require weighing again if the period was only a few days.

The Mississippi field fertilizer tests have a check plot after each three plots. The yields of the check plots were interpolated to determine the check yields of the plots between the checks. The increases in carbon dioxide obtained in the laboratory as a result of the addition of nitrogen and of nitrogen plus phosphorus were compared with the following field tests: 600 pounds 6–0–0 and 600 pounds 6–8–0, except in the case of Houston clay where the nitrogen was 8 per cent instead of 6. Both the field and laboratory data are reported as increases over no fertilizer. The no fertilizer yields of seed cotton and the carbon dioxide produced without fertilizer are also reported. The laboratory data reported are usually an average of duplicate determinations. The agreement of the duplicate determinations usually approach that of duplicate quantitative chemical determinations. The increase for phosphorus was obtained by subtracting the increase for nitrogen from the increase for nitrogen plus phosphorus in both the field and laboratory data. No response was obtained for muriate of potash in the laboratory under any conditions, and very little was obtained for phosphorus when added without nitrogen.

<div align="center">RESULTS</div>

The Mississippi soils were collected in July and air dried, and the laboratory determinations were made soon afterwards. The odds were calculated for the field data by the use of Student's method. The data for the Mississippi soils are reported in table 1 and figure 1. The scale used in figure 1, 1 mgm. of CO_2 = 4 pounds of seed cotton, was chosen so that the lines representing increases in seed cotton and those representing increases in CO_2 would fall as closely together in the figure as possible. In setting up an arbitrary scale of this kind the assumption is made that the factors other than nitrogen and phosphorus have been equally distributed in all cases, but it is quite well known that nature does not do things that way when only a few years are involved.

Orangeburg fine sandy loam.—The field test was conducted only 1 year. On this year the increases due to fertilizer were larger than would be obtained over a 5-year average. When the data are considered as they stand, nitrogen produced an increase of 559 pounds of seed cotton and phosphorus produced an increase of 33 pounds; the increases in CO_2 in the laboratory indicate increased yields

TABLE 1

The response of cotton and soil microörganisms to nitrogen and to phosphorus

| SOIL TYPE | LOCATION | pH* | YIELD DATA‡ | Yield, no fertilizer | LBS. SEED COTTON PER ACRE | | | | | Yield without fertilizer | MGM. CARBON DIOXIDE | | |
| | | | | | Increase | | Increase | | In-crease | | | Increase | |
					N over none	Odds	NP over N	Odds	N and P over none		N	NP over N	NP
Houston clay	Prairie, Miss.	6.7	12	501	.8	1:1	385	1110:1	393	51	9	70	79
											82	23	105
Ruston fine sandy loam	Columbus, Miss.	6.5	12	290	397	9999:1	174	9999:1	571 {	November 22 July 25	54	52	106
Orangeburg fine sandy loam	Macon, Miss.	7.4	2	230	559	24:1	33	4:1	592	32	112	7	119
Norfolk fine sandy loam	Macon, Miss.	6.1	9	560	179	9999:1	194	2999:1	373	28	80	49	129
Oktibbeha clay	Macon, Miss.	5.9	6	343	26	3:1	297	1666:1	323	46	23	44	67
Sarpy fine sandy loam	Stoneville, Miss.	6.2	15	820	388	9999:1	−1	......	287	46	115	15	130
Trinity clay	Sessums, Miss.	7.8	12	520	303	9999:1	77	142:1	380	61	112	7	119
Denham silt loam	Baton Rouge, La. †	5.9	20	978	475	9999:1	50	14:1	525	47	87	16	103
Ruston fine sandy loam	Calhoun, La.	6.4	20	953	358	9999:1	145	188:1	503	28	98	7	105
Yohola very fine sandy loam	Alexandria, La.	6.8	6	1443	397	260:1	125	4:1	522	51	90	14	104
Ruston fine sandy loam	Homer, La.	6.3	15	716	202	5999:1	138	192:1	340	15	55	32	87
Ruston fine sandy loam	DeRidder, La.	6.0	6	612	505	4699:1	238	195:1	743	22	40	30	70
Memphis silt loam	Gurley, La.	5.8	8	254	71	10:1	409	3332:1	480	42	7	18	25

* The pH was determined colorimetrically using Hellige color discs.

† The Louisiana soils had 4 per cent potash applied in the field in addition to the nitrogen and phosphorus treatments.

‡ Replications.

of seed cotton of 448 pounds and 28 pounds respectively. In both the field and the laboratory very large increases were obtained from nitrogen alone and very small increases from phosphorus.

Ruston fine sandy loam.—This test was conducted for 4 years in the field. The increases in seed cotton for nitrogen and phosphorus were 397 pounds and 174 pounds respectively; the increase in CO_2 indicates increases of 328 pounds
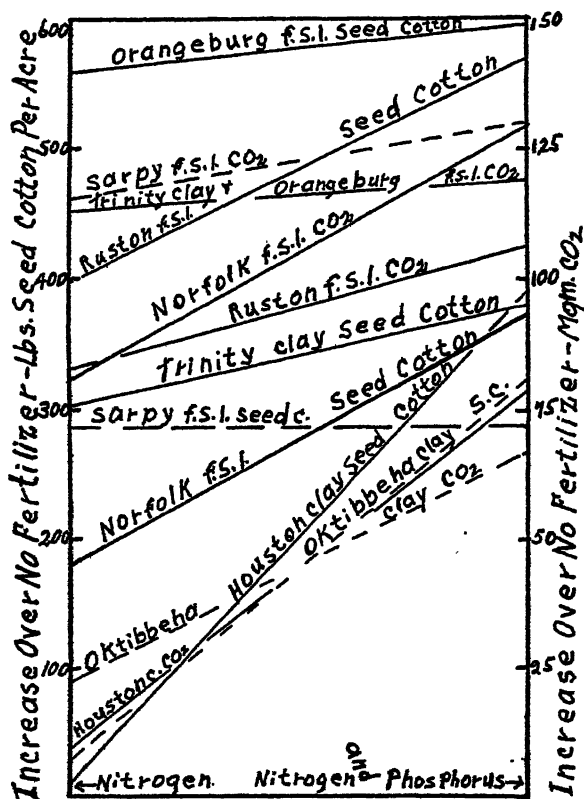
FIG. 1. RESPONSE OF COTTON AND SOIL MICROÖRGANISMS TO NITROGEN AND TO NITROGEN AND PHOSPHORUS—MISSISSIPPI SOILS

Scale—1 mgm. CO_2 = 4 pounds of seed cotton

and 92 pounds respectively. These differences are considered to be within the experimental error of the field results.

Norfolk fine sandy loam.—The field test was conducted 3 years. The increase obtained for nitrogen in one year which was extremely dry was 74 pounds per acre, whereas the average for the other 2 years was 231 pounds. The average increase for nitrogen for the 3 years was 179 pounds. The increase in CO_2 in the laboratory due to the addition of nitrogen indicates an increase in seed cotton of 320 pounds per acre. Phosphorus increased the yield of seed cotton

194 pounds; the increase in CO_2 obtained in the laboratory indicates an increase of 196 pounds of seed cotton for phosphorus.

Trinity clay.—The field data are reported for 3 years. The increases in seed cotton were 303 pounds and 77 pounds of seed cotton per acre for nitrogen and phosphorus respectively. The increases in CO_2 obtained indicate increases in yields of 448 pounds and 28 pounds of seed cotton per acre for nitrogen and phosphorus respectively.

Sarpy fine sandy loam.—The field data reported are for 5 years. Nitrogen made an increase in the yield of seed cotton of 288 pounds, whereas the increase in CO_2 produced indicates an increase in yield of seed cotton of 460 pounds, a difference of 172 pounds. This is the largest difference found in any of the tests, and it would be difficult to attribute all of the difference to field experimental error, but it is well to consider that the average increase in the seed cotton reported is derived from individual increases which vary from 61 to 740 pounds per acre. The addition of phosphorus did not increase the yield of seed cotton, whereas the increase in CO_2 obtained indicated an increase in seed cotton of 60 pounds. This difference is considered to be within the experimental error of the field work.

Houston clay.—This test was conducted for 3 years in the field. The nitrogen applied in the field was 8 per cent instead of 6 per cent as in the other tests. The same quantity of nitrogen was used on this soil in the laboratory as on the other soils. If the quantity of nitrogen used in the laboratory had been increased sufficiently to correspond with the amount applied in the field, the increase in CO_2 for nitrogen and phosphorus would have been somewhat larger. The lines representing increases in CO_2 and seed cotton would have almost coincided, indicating perfect agreement between the two methods.

Oktibbeha clay.—The field data are for 2 years. The increase for nitrogen and phosphorus together is 323 pounds; the increase in CO_2 indicates an increase in seed cotton of 268 pounds. The difference between the two methods (55 pounds) is considered to be within the experimental error of the field work. The increase per acre for nitrogen was 26 pounds of seed cotton, an average of 59 pounds on one year and −7 pounds on the other. The increase in CO_2 indicated an increase of 92 pounds of seed cotton for nitrogen. This difference is also considered to be within the experimental error of the field work.

The treatments in the Louisiana field tests which were used in comparison to the data obtained in the laboratory were:

Plot No. 1 600 lbs. 5–0–4
Plot No. 2   —   —
Plot No. 3 600 lbs. 5–8–4
Plot No. 4   —   —
Plot No. 5   —   —
Plot No. 6   —   0–0–0

Student odds were calculated on the assumption that the fertility of the soil between plots 1 and 6 is uniform. The soil for the laboratory work was col-

lected the middle of August; the laboratory determinations were made 3 months later. The data are reported in table 1, and those for four soil types are illustrated in figure 2.

Denham silt loam.—The field test was conducted for 5 years. The increase for phosphorus obtained in the field was 50 pounds per acre; the increase in CO_2 indicates an increase in yield for phosphorus of 64 pounds. The increase for nitrogen obtained in the field was 475 pounds, whereas the increase in CO_2
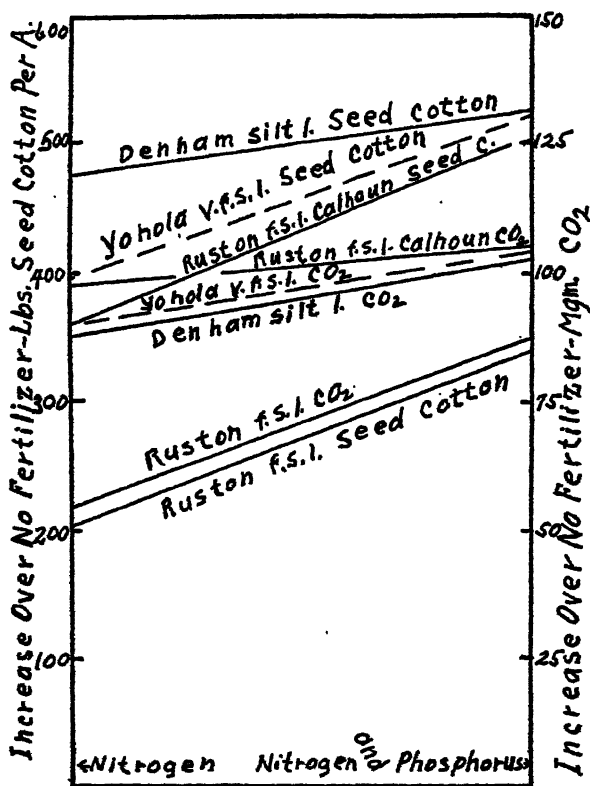
FIG 2. RESPONSE OF COTTON AND SOIL MICROÖRGANISMS TO NITROGEN AND TO NITROGEN AND PHOSPHORUS—LOUISIANA SOILS

indicated an increase of 348 pounds. The difference of 127 pounds per acre is very probably due to the control of boll weevils on this test, whereas on most of the other tests they were not controlled artificially.

Yohola very fine sandy loam.—This test ran for 2 years. The laboratory and the field method gave approximately the same response to nitrogen. The laboratory method gave an increase in CO_2 for phosphorus, which indicates an increase in seed cotton of 54 pounds per acre; 125 pounds with odds of only 4 to 1 were obtained in the field. The odds indicate that the increase obtained in the field was not due to the phosphorus.

Ruston fine sandy loam, Calhoun.—The field data are for 5 years. The increase in seed cotton for nitrogen obtained in the field was practically the same as that indicated by the increase in CO_2. The increase for phosphorus was 145 pounds of seed cotton per acre; the increase in CO_2 indicates an increase for phosphorus of only 28 pounds of seed cotton.

Ruston fine sandy loam.—The increases obtained in the field over a 4-year period were practically the same as those indicated by the increases in CO_2.

Ruston fine sandy loam from De Ridder, and Memphis silt loam.—The laboratory data indicate increases in seed cotton very much smaller than those obtained in the field. The differences are so great that the conclusion is reached that there is very little agreement in the data obtained by the two methods. The question naturally arises: "What is the lack of agreement due to?" It should

TABLE 2

The effects of air-drying soil for 24 hours and for 4 months and of the time of the year during which the soil is collected on the response of the soil microörganisms to nitrogen and to nitrogen and phosphorus

SOIL TYPE*	SOIL SAMPLE			MGM. CARBON DIOXIDE			
				Yield, no fertilizer	Increase for		
	Date taken	Date run	Condition		N	NP over N	NP
	1933	*1933*					
Oktibbeha clay................	July	July	Air-dry	46	23	44	67
Oktibbeha clay................	July	Nov. 3	Air-dry	47	2	11	13
Oktibbeha clay................	Nov. 28	Dec. 1	Air-dry	39	27	14	41
Oktibbeha clay................	Nov. 28	Dec. 1	Moist	34	13	12	25
Orangeburg fine sandy loam.....	Nov. 28	Nov. 30	Moist	20	128	2	130
Orangeburg fine sandy loam.....	Nov. 28	Nov. 30	Air-dry	22	125	4	129
Orangeburg fine sandy loam.....	July	July	Air-dry	32	112	7	119
Orangeburg fine sandy loam.....	July	Nov.	Air-dry	42	114	11	125

* All soils taken from Macon, Miss.

be borne in mind that 3 months elapsed between the time the soil samples were collected and the time the laboratory determinations were made.

The effect of storing air-dry soil from July until November on the CO_2 production.—In order to throw some light on the foregoing apparent disagreement between the two methods, Mississippi soils which were collected in July were used for laboratory determinations in November. The data are reported in table 2. Practically the same increases in CO_2 were obtained for fertilizers applied to the Orangeburg soil in July as in November, whereas there is no resemblance between the data for the July and November increases in CO_2 for the Oktibbeha soil.

Evidently air-drying for 4 months produced little change in the activity of the soil microörganisms of the Orangeburg soil, whereas much change took place in the activity of the soil microörganisms of the Oktibbeha soil.

The effect of air-drying soil for 24 hours on the production of CO_2.—Orangeburg and Oktibbeha soils were obtained from the fields in November. Determinations were made on fresh moist soil and on the soil after drying 24 hours. The data are reported in table 2. Air-drying did not materially affect the activity of the soil microörganisms in the Orangeburg soil as measured by CO_2 production but it increased markedly the activity of the soil microörganisms of the Oktibbeha soil.

The effect of the time of the year during which the soil is collected on the production of CO_2.—Soils were collected in November from several fields from which samples had been collected in July. The data for the Orangeburg and the Oktibbeha soils, reported in table 2, show that the CO_2 produced by the soil microörganisms of the Orangeburg soil was practically the same in November as in July, whereas there was a marked difference in the CO_2 produced by the microörganisms of the Oktibbeha soil when collected on the two dates. The laboratory data for all soils collected and run in July agreed with the field data, whereas the laboratory data for the November collected soils were very variable.

DISCUSSION

Soil microörganisms require approximately the same nutrients as do crop plants. The plants obtain energy from the sun, whereas soil microörganisms require the presence of an energy-supplying substance. The activity of the soil microörganisms depends upon the available nutrients and may be measured by determining the evolution of CO_2. The response of the soil microörganisms of a particular soil to nitrogen and to nitrogen plus phosphorus may be determined by measuring the increase in CO_2 produced by the addition of these fertilizers to mannite-treated soil. The results obtained in these experiments show that there is a high correlation between the response of soil microörganisms and cotton to nitrogen and to nitrogen plus phosphorus. Soil samples were obtained from 13 fields in which fertilizer experiments had been conducted for cotton, and the response of the soil microörganisms to nitrogen and phosphorus were obtained in the laboratory.

In 10 out of 13 cases the increase in CO_2 due to the addition of phosphorus plus nitrogen indicated increases in yields of seed cotton which came close enough to that actually obtained in the field to be within the range of the experimental error of the field data. In 9 out of 13 cases the increase in the CO_2 due to the addition of nitrogen came close enough to that actually obtained in the field to be within the range of experimental error of the field results. In 2 cases out of 13 the increase in yield of seed cotton from nitrogen alone was very much less than that indicated by the increase in the production of CO_2 in the laboratory.

The differences found between the two methods in the case of nitrogen are considered to be due to the irregularity in the distribution of those factors which favor cotton production.

In 2 cases out of 13 there was little correlation between the increases in CO_2 and seed cotton due to the addition of fertilizers. Further tests showed that this very probably was due to changes having taken place in the activity of the soil microörganisms during the lapse of 3 months between the collection of these soil samples and the laboratory determination.

The increase in CO_2 due to the addition of fertilizers to soils in July was in every case in direct proportion to the increases in seed cotton obtained in the field, whereas when the soil was collected in November in some cases there was almost no correlation between the field and the laboratory method.

Air-drying of soil collected in November was found to change markedly the activity of the soil microörganisms of some soils.

Carbon dioxide production on mannite-treated soil as a means of determining crop response to fertilizers has the following advantages over field tests:

1. Less than 30 hours is required to complete a test.

2. After the sample of soil reaches the laboratory, the actual time required to carry out the work is less than 3 hours.

3. The cost is sufficiently low to permit colleges to put in service departments to determine the fertilizer response of soils on which it is absolutely impossible to carry out field fertilizer experiments.

CONCLUSION

The production of CO_2 in soils to which mannite has been added under controlled laboratory conditions furnishes a basis for measuring the nitrogen and phosphorus requirements of soils for cotton. The nitrogen and phosphorus requirements of soils for other crops may be determined by this method as soon as the laboratory data have been properly correlated with sufficient field data.

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THE FACTORS WHICH INFLUENCE THE USE OF THE CONDUCTIVITY OF SOIL SUSPENSIONS AS A MEASURE OF FERTILITY

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The most fruitful application of conductivity measurements to the study of the soil has so far undoubtedly been the determination of total salts in saline deposits. As far back as 1897 Whitney and Means (22) evolved a quick electrolytic method for the determination of total salts in soils, which was further elaborated and improved upon by Briggs (3), Cameron and Briggs (4), Davis and Bryan (8), and others, until today the portable Wheatstone bridge has become an indispensable instrument to the soil surveyor engaged in the mapping of saline soils for purposes of reclamation or land utilization.

Atkins (1), however, was among the first to suggest conductivity as a measure of soil fertility. He showed that the conductivity of aqueous soil suspensions increased with time of extraction, tending towards a maximum value. Although some soils reached this maximum in a few hours, others continued to increase even after 10 to 14 days of continued extraction. CO_2, bacterial activity, and the solution of the soil material itself were suggested as factors influencing this change. He states: "A high electrical conductivity in the extract may only indicate the presence of excess salts and does not necessarily indicate a good soil. It seems however that a rapid increase as extraction is prolonged indicates increased solubility partly through bacterial action and may be considered as a useful indication of fertility. A low conductivity, which remains low on continuous extraction, denotes a soil so insoluble as to be infertile." Davis (7) confirmed Atkins' results.

Sen (18), employing a more standardized method of determination, e.g., excluding atmospheric CO_2 and using a constant temperature and a soil-water ratio of 1:5, found that the conductivity increase after 8 to 10 days bears a close relation to the fertility of the soil as checked by crop yield and crop growth. Fertile soils invariably showed large conductivity increases, infertile ones, slight or no increases. He states that of two soils practically identical in chemical composition but differing widely as regards crop response, the fertile one showed the highest conductivity increase, and concludes that "conductivity measurements are capable of accounting for failure to grow crops where chemical analysis yields no indication."

Sen and Wright (20) applied the foregoing method to the study of soil samples taken from the Rothamsted fertility plots over a long period of years. Choosing the conductivity increase over a period of 7 days—"the 7 days' increase"—as an arbitrary maximum for comparison, they came to the following conclusions: On prolonged storage the initial conductivity is not markedly increased, but the 7 days' increase rises rapidly in a few months to a constant maximum value.

Manurial treatment influences both the initial conductivity and the 7 days' increase, and there exists a high positive correlation (0.859) between 7 days' increase of stored samples for various years and the crop yields for those years. If the time factor is eliminated, however, by calculation of a partial correlation, the value found is insignificant (+.061). When soil in a low state of fertility is allowed to run wild it shows an increase both in initial conductivity and 7 days' increase, which is in accord with normal cropping experience.

Sen (19) further studied the influence of cultivation, manuring, cropping, and meteorological variations on the cropped and uncropped Rothamsted plots. Although no seasonal change in unmanured plots of low fertility is evident, it is extremely marked in soils under permanent grass. To correlate 7 days' increase with fertility, measurements must be carried out when soil is in normal equilibrium, i.e., not shortly after the application of manures or the plowing in of stubble. A large 7 days' increase will then indicate that the soil is favorable to biological activities and therefore to the rapid liberation of plant food. Such a soil may therefore be expected to have a higher inherent fertility than one which has a smaller 7 days' increase.

Benade (2) has contributed a very interesting series of observations on the factors which influence conductivity increases in soils. The most important among these are (a) CO_2 absorption, (b) bacterial activity, (c) solution of the soil material, the quantitative contribution of each of which to the total increase was measured. As these results will be discussed in greater detail later, they will not be further elaborated here.

Judging from the evidence in hand, there would therefore appear to exist a general relation between conductivity increase in soils and crop response. The fundamental reasons for such a correlation are, however, as obscure as they are surprising, and the present paper is a further inquiry into the validity of these apparent relationships.

METHOD OF DETERMINING CONDUCTIVITY

Conductivity measurements were carried out by means of a Leeds and Northrup slide wire instrument, oscillator, and telephone, using a dip cell (cell constant = 1.04), conductivity water ($C = 15 \times 10^{-5}$), and a soil-water ratio of 1:5. The results are expressed as $C \times 10^{-6}$. The determinations were carried out in much the same way as suggested by Sen and Wright (20): 40 gm. of 2-mm. air-dry soil was placed in a resistant bottle, 200 cc. conductivity water added direct from fused silica receiver of conductivity water apparatus.

The suspension was agitated by an electrical stirrer, with the dip cell in position and in a current of CO_2-free air, the whole being carried out in a thermostat at 25°C. Measurements were taken every few minutes until the conductivity remained constant for 10 to 15 minutes. This value was the initial specific conductivity at 25°C. The suspension was left in the thermostat for 7 days, and the conductivity was again determined. Immediately before each determination the stirring was stopped for 100 seconds, in which time both the coarse and fine sand had dropped below the level of the dip cell, which was always immersed to a constant depth with respect to the surface of the liquid. The reason for this procedure will become clear from the discussion later.

The method of sampling is another very important item in measurements of this kind and can lead to serious variations in the same soil. This matter was given particular attention in these experiments, and no samples were used as duplicates or blanks until sampling could be carried out in such a way as to give the same initial conductivity.

EXPERIMENTAL RESULTS

The factors which influence conductivity fluctuations in soil suspensions can be grouped under the following heads: (a) adsorption of CO_2 from the atmosphere; (b) the action of microorganisms; (c) change in the degree of dispersion of the solid phase. Benade makes reference to these three, but there is also a fourth factor, (d) the formation of simple electrolytes as the result of solution of easily soluble salts, and the formation of colloidal electrolytes by hydrolysis of the colloidal complex.

To obtain information on the value for the initial specific conductivity, the following experiment was conducted.

It is a well-known observation that when soil is placed in water, the specific conductivity increases rapidly, a state of temporary equilibrium representing the solution of easily soluble salts being reached within from 15 minutes to somewhat over an hour, depending on the soil texture and the method of agitation used. After this point the conductivity remains practically constant, increasing relatively slowly with continued extraction, as a result, as will be seen later, of bacterial activity and hydrolytic changes in the soil complex. The first apparent maximum is a very well-defined point for all soils and is called the "initial conductivity." Thus:

Time..........	2 min.	5 min.	10 min.	19 min.	23 min.	28 min.	38 min.	24 hrs.	7 days
$C \times 10^{-6}$.......	67.0	73.9	120.4	185.6	199.8	203.6 ("Initial conductivity")	203.6	209.0	215.0

"Seven days increase" = Conductivity attained in 7 days, initial conductivity.
= $215 - 203.6 = 11.4 \times 10^{-6}$

The action of Microörganisms

Any microbial activity in a soil completely immersed in water, as in the "7 days' increase" determination, must of necessity be greatly influenced by the artificial environmental conditions. If an adequate food supply is assumed, the aerobic organisms would be active from the start until such time as the high CO_2 concentration and consequent reduction in oxygen tension would cause them to be displaced first by facultative and finally by obligate anaerobes. Aerobic activity is hardly likely to last more than a few days under these conditions. On the other hand, it is well known that anaerobic forms require a considerable period of incubation for maximum development—up to 11 days under favorable circumstances. The strong presumption therefore exists that the initial aerobic activity is the only microbial contribution to be reckoned with and that the latter portion of the 7 days incubation period must be one of almost complete microbial inactivity. Evidence in support of this view is presented in the following.   .

If these arguments are correct, one must conclude that the CO_2 concentration of the liquid phase as influenced by the aerobic organisms is the limiting factor in microörganic activity, for only in extremely exceptional circumstances will a soil material be found so devoid of nutrients as to make this a limiting factor in an experiment of this kind. As the soil:water ratio in these determinations is constant (1:5), the feebler microbial activity in a poor soil will proceed for a longer time than that in a fertile one. Both will cease, however, when the same maximum CO_2 concentration has been reached.

The conductivity increase due to microbial action can therefore bear no relation whatsoever to the microörganic activity of the soil in its natural condition. The microörganisms under these conditions merely act as agents to produce a certain more or less constant CO_2 concentration, the whole process being analogous to shaking up a soil with water containing a fixed amount of CO_2, a method employed at various times in different parts of the world for determining available plant food.

If this is true, the increase due to organisms should bear a nearly constant ratio to the total increase whatever the nature of the soil. Soils so unweathered as to be entirely devoid of nutrients or those containing toxic elements, as for example excess of soluble salts, will be exceptions. In a series of quantitative studies on soil sterilized (with CS_2) and unsterilized, Benade (2) showed that the total increase is the resultant of microbial action and a solution effect on the soil body itself. His figures recalculated to show per cent increase due to bacteria are presented in table 1. From this it is clear that for these soils of different types the increases due to bacteria are practically constant, i.e., about 47 per cent of the total. For the South African soils examined it amounted to 50 per cent.   ˉ

In order to test these points further, the following procedure was followed: Eight 40-gm. 2-mm. samples were taken from the same soil in such a way that their initial conductivities were identical, and the dry soil was placed in

resistant flasks and plugged loosely with cotton wool. Four of these were sterilized at 230°C. and 9 atmospheres pressure for 3 hours. To each of the eight flasks, 200 cc. freshly boiled and cooled conductivity water ($C = 16$ x 10^{-5}) was added by means of a sterilized pipette, the same precaution against infection being taken as is normal in bacteriological work. The flasks were immediately stoppered, shaken, and placed in a thermostat at 25°C. When equilibrium had been attained (after 48 minutes determined by preliminary experiments) one sterile flask and one non-sterile flask were opened and the conductivities determined. This represented the initial conductivities for the purpose of correcting for any change in conductivity caused by the process of sterilization. After 2, 5, and 7 days this procedure was repeated. Results are presented in table 2 and graphically in figure 1.

It might be mentioned here that the procedure was repeated on the same soil on three different occasions, giving practically identical results, indicating that the sterilization and method of experimentation had been refined to the required degree of accuracy.

TABLE 1

Microörganisms and conductivity

SOIL TYPE	28 DAYS CONDUCTIVITY INCREASE		INCREASE DUE TO ORGANISMS	PER CENT OF TOTAL
	Sterile	Non-sterile		
	$C \times 10^{-4}$	$C \times 10^{-4}$	$C \times 10^{-4}$	
Sandy soil (1.2 per cent humus)...........	42.9	91.7	48.8	53
Drift sand (1.34 per cent humus)..........	66.3	121.4	55.1	45
Clay soil (2.67 per cent humus)...........	270.8	479.8	209.0	44

The results show that: (*a*) Microörganic activity practically ceases between the third and fourth day of incubation; (*b*) Further increases are due to hydrolytic and solution effects on the soil material; (*c*) The pH increases to nearly the same extent during incubation both in sterile and non-sterile soil. Benade, who found a similar pH increase, ascribed it to reduction processes under anaerobic conditions and the consequent production of NH_3. A more likely reason, however, is the hydrolysis of the complex, the dissociation of adsorbed bases, and the consequent increase of OH ions. In the presence of CO_2 this effect would probably be enhanced as the result of formation of bicarbonates. The point will, however, be discussed in greater detail later.

CONDUCTIVITY CHANGES DUE TO DEGREE OF DISPERSION OF THE SOLID PHASE

With regard to the influence of the soil particles themselves on the conductivity of a suspension, it has been pointed out before that the time taken for the attainment of initial equilibrium ("initial conductivity"), though usually greater for clayey than for sandy soils, would obviously depend on the state of aggregation of the soil particles.

Whitney and Means (22) many years back reported an illuminating series of observations on this point. They added increasing quantities of pure sand to the same volume of solution and found that the resistance increased slowly

TABLE 2

Microörganisms and conductivity

	STERILE		NON-STERILE		INCREASE DUE TO MICROBES	INCREASE DUE TO SOLUTION
TIME	$C \times 10^{-6}$	pH	$C \times 10^{-6}$	pH	$C \times 10^{-6}$	$C \times 10^{-6}$
days						
0	164.2	5.55	143.2	5.38	....	....
2	178.2	5.59	169.1	5.43	11.9	14.0
5	182.0	6.08	184.4	5.76	23.0	17.8
7	183.5	6.14	185.8	6.23	23.3	19.3
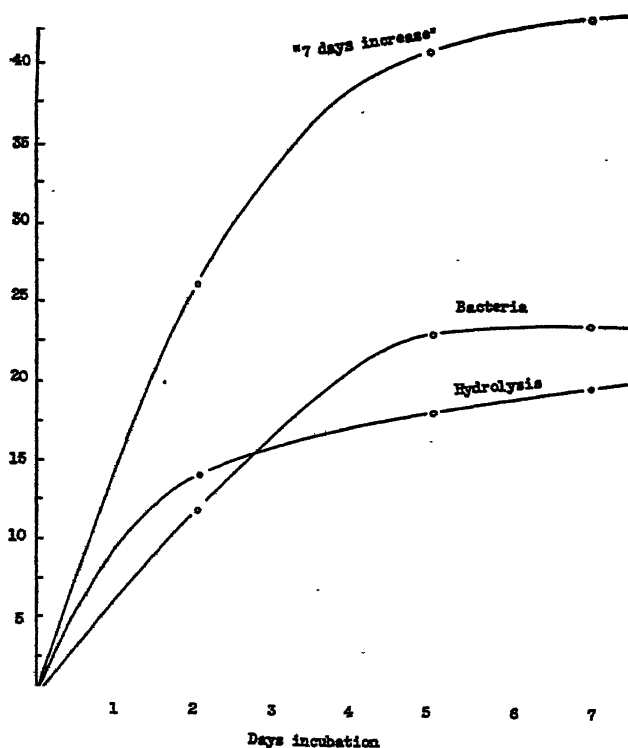
FIG. 1. CONTRIBUTING FACTORS IN "7 DAYS INCREASE"

up to the point of complete saturation and after that very suddenly, resulting into two straight lines intersecting at the point of complete saturation of the soil by water. Beyond this point air would begin to fill the pores and with decreasing moisture content the thickness of the films round the particles

would continuously decrease. The effect of this on ionic mobility would be analogous to a lengthening of the path between the electrodes. With soils of different texture, adjusted to the same salt and moisture content (below the point of complete saturation), the resistance increased with increasing surface area, i.e., with decreasing thickness of the moisture film. In the same way a soap solution had a higher specific conductivity than an equivalent amount of foam.

Haines (10) and Cashen (5), following up the work of the Bureau of Soils, have used conductivity measurements to study possible discontinuities in the soil-moisture curve below the point of complete saturation. Cashen points out that the net effect of the movement of ions, which are naturally more concentrated at the soil-water interface, is similar to the passage of an alternating current through a condenser resulting in a phase difference between the applied E. M. F. and the current flowing. His apparatus is designed to measure such capacity changes in a system decreasing in moisture content. Haines has pointed out, however, that drying has to be very slow in order to obviate differences in moisture content between different parts of the soil block. A drying process lasting over several days would introduce changes in the electrolyte content, both as the result of bacterial action and changes in the colloidal complex on the one hand, and a reduction in the moisture film on the other. The conductivity at any particular moisture content must therefore be the resultant of a series of opposing forces, on the one hand an increase in electrolyte content, as well as surface conductance effects tending to cause increased conductivity, and on the other a decrease in the thickness of the moisture film tending to decrease the conductivity.

Cashen appears to have looked upon the soil as a more or less inert aggregation of particles surrounded by films of constant electrolyte concentration, and has disregarded almost entirely any physico-chemical changes accompanying the process of desiccation. It is difficult to see how his figures can reflect true discontinuities in the soil-moisture curve with any degree of accuracy.

Above the point of complete saturation inert particles like sand will have little or no effect on ionic mobility, so that at a 1:5 soil-water ratio any such influence can be entirely disregarded. If the conductivity of a soil suspension is therefore determined by ionic mobility alone, it should be unaffected by the coarser particles, e.g., sand and fine sand. To test this point, different soil suspensions (1:5) were stirred at 25°C. (by means of an electric stirrer) until initial equilibrium was attained, the dip cell being in position at a fixed depth. At this point the stirring was stopped and the coarse and fine sand allowed to drop below the electrodes—under the given set of conditions constancy was obtained in 100 seconds—and the conductivity again measured. The suspension was then centrifuged for 45 minutes and the determination repeated (table 3).

From these results it would appear that the conductivity is increased as the

coarser particles are removed, first by sedimentation and then by centrifuging. The centrifuged material was a translucent sol and the only obvious explanation of the phenomenon is that the finest sol particles must act like ions and contribute to the conduction of the current, with mobilities not very far removed from that of the ions themselves. Although the coarser suspended material has no influence on the mobilities of the ions, they very definitely retard the movement of these complex ions, thus causing an increase in conductivity when the mechanical obstruction is removed.

Soils A and B, respectively a ferruginous and highly silicious clay, behaved somewhat differently on centrifuging. Here the total number of dispersed

TABLE 3

Influence of coarse particles on conductivity   ·

STIRRING TIME	SOIL NUMBER							
	364 $c \times 10^{-?}$	404 $c \times 10^{-?}$	373 $c \times 10^{-?}$	355 $c \times 10^{-?}$	371 $c \times 10^{-?}$	444 $c \times 10^{-?}$	A $c \times 10^{-?}$	B $c \times 10^{-?}$
minutes								
2	146.9	180.3	162.5	30.2	.....	142.0	112.7	93.0
5	149.7	184.9	165.2	.....	73.9	145.3	115.4	97.9
10	151.6	192.9	170.0	31.7	120.4	148.4	118.7	106.5
15	152.2*	200.7	170.2	.....	160.0	150.2	129.0	114.7
20	152.2	208.7	170.2	32.8	190.0	151.4	122.5	122.7
30	152.2	221.5	170.2	33.6	203.6	152.9	124.9	137.0
35	152.2	222.3	170.2	34.7	203.6	.....	125.5	137.0
50	152.2	222.3	170.2	35.4	203.6	154.7	126.9	137.0
60	152.2	222.3	170.2	35.4	203.6	155.2	126.9	137.0
70	152.2	222.3	170.2	35.4	203.6	155.8	126.9	137.0
80	152.2	222.3	170.2	35.4	203.6	155.8	126.9	137.0
Sedimentation for 100 seconds...	163.5	225.0	184.4	36.5	218.0	162.1	134.2	145.0
Centrifuged 45 minutes.........	173.2	228.7	213.1	37.9	220.2	168.9	130.5	132.4
Per cent clay.................	.....	.....	10.9	13.0	19.0	22.0	50.0	50.0

* Figures in boldface type represent the "initial conductivity."

particles is large and some are obviously carried down by the coarser material, causing a decrease in conductivity on centrifuging. These results seem to be in agreement with those of McBain (11), who found in his work on soap solutions that if the fatty acid chain is increased until it attains a size incommensurate with molecular dispersion the particles formed have mobilities of the same order as that of ions.

A fairly close relation therefore appears to exist between conductivity, electrophoresis, and surface conductivity, and Pugh (16), in a discussion on electrokinetic potentials in the light of the work of Michaelis (14), McBain (11), Loeb (15), and in particular that of Mattson (12), presents a summary of these

views. In solution the colloidal micelle with its double layer can be considered in many respects similar to the molecule of an electrolyte. There is an analogous space differentiation between anionic and cationic components, but, although the ions in electrolytes distribute themselves nearly uniformly through the whole solution, in the case of the micelle, electrostatic effects cause the cations, which though free to move amongst themselves, to be arranged in the nature of a diffuse swarm around the complex anion. Under the influence of an electric field, anions and cations move in opposite directions, so that visible movement of the anionic micelle, which is known as cataphoresis in the case of electronegative colloids, can therefore be regarded as a limiting case of conduction in electrolytes.

The transition from a molecularly dispersed to a colloidally dispersed state is one of degree and represents a change from a condition where electrostatic attractions are infinitely small to one where they are highly marked, with an intermediate zone where particles show the properties of both extremes and mobilities approaching those of individual ions. Such particles would contribute to the conduction of a current and influence conductivity measurements. With increase in size and consequent reduction in mobility the bigger particles, although capable of cataphoretic movement, will obstruct the movement of the transitional micelles.

These arguments naturally apply to dilutions where the particles have comparatively free movement. If the number of particles is increased to a point where individual movement ceases, as in a gel or a block of damp soil, endosmotic effects become dominant and the cationic movement of the adsorbed ions manifests itself as surface conductance.

HYDROLYSIS OF THE COMPLEX

One of the outstanding properties of weak electrolytes is their tendency to hydrolyze, which is now generally recognized as due to the higher affinity of the H ion for the weak acid ion as compared with other cations. The reaction, which can be represented thus:

$$XM + H_2O \rightleftharpoons XM + H' + OH' \rightleftharpoons XH + M' + OH'$$
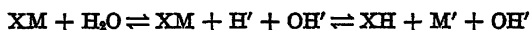
therefore gives rise to OH ions and cations, the solution tending to become alkaline.

The mineral silicates as well as the soil complex are salt-like substances consisting of an anionic and cationic component, which in water behaves much like weak electrolytes. Thus powdered feldspar when shaken up with water will yield cations and an increased pH, whereas analyses of weathered and unweathered rocks like granite show a considerable loss of cations in the weathered material. In fact this reaction is regarded as one of the most potent factors in the weathering of rocks and soils, and dealkalization as the first step in this process (20).

Although, because of the amphoteric nature of soil colloids, conductivity

measurements have little or no meaning in physico-chemical studies on them, they can be used as a relative measure of the total amount of basic material dissociated when a given soil is treated with a given amount of water.

In the absence of many soluble salts the action of water on soil material would be largely one of hydrolysis and increased dispersion. Drachev (9) has attempted to measure this hydrolysis by means of conductivity determinations assuming that Ostwald's equation for weakly associated binary electrolytes holds for soil colloidal micelles, i.e.

$$\frac{a^2}{(1-a)v} = K \text{ or } a = \sqrt{KV}$$

in the case of soils where dissociation is small (a = dissociated part of molecule, v = volume of solution). By plotting milligram equivalents electrolyte dissociated against dilution, he obtained a series of characteristic straight lines from which a solubility factor for different soil types could be deduced. There are, however, a number of discrepancies in Drachev's method of treatment. For example, he does not indicate how he reduces conductivities in solutions of mixed ions to milligram equivalents electrolyte, which on the face of it must entail certain assumptions and approximations. In the second place, for conductivity measurements applied in this way to have any meaning at all, they must be carried out on equivalent concentrations of colloid. Cziky (6) has pointed this out in comparisons of base exchange capacity, and it certainly applies with equal force here. To compare hydrolysis of a chernozem complex with that of a podzol, therefore, it is necessary to use equivalent quantities of colloid as regards base exchange capacity. The different solubilities found by Drachev may therefore either all have been equal or of an entirely different order, had the colloidal materials been used in equivalent concentrations. As has been pointed out, however, the validity of applying conductivity determinations in this way can be questioned on the grounds of both the amphoteric nature of the colloid and the influence of dispersion on conductivity.

In the following experiment the relation between dispersion and increase in conductivity was tested. Soils differing in pH and clay content were boiled with conductivity water (1:5) under a reflux condenser, thus excluding CO_2 and bacterial action, for various periods. At different periods the change in conductivity and dispersion was determined. From the results given in table 4, it will be seen that both the conductivity and the degree of dispersion increase with boiling. The rate of this increase differs from soil to soil, but as equivalent concentrations of complex were not taken, this does not reflect different degrees of hydrolysis of the complexes, but that of the whole soil body, thus including the diluting effect of the sand and coarser particles, as is normally the case in determining available plant food in the laboratory.

In most soils there is a rapid conductivity increase during the first few hours. After that, however, increases with time are comparatively slight (fig. 2). In a few instances soils like 371 and 367 were found which increased at a nearly

constant rate almost indefinitely, a difference which is by no means always reflected by ordinary chemical analysis.

If the degree of dispersion is plotted against conductivity increase (fig. 3), it would appear that conductivity increases in a fairly regular manner with increasing dispersion, in some cases actually resulting in a straight line, but it is somewhat more rapid than change in dispersion. Such a close relation is naturally to be expected, for with an increase in the number of finer particles an increased surface is exposed to the effects of hydrolysis. In some soils, however, though a limit is reached in dispersion, conductivity increases continue, showing the essentially independent nature of the two processes.
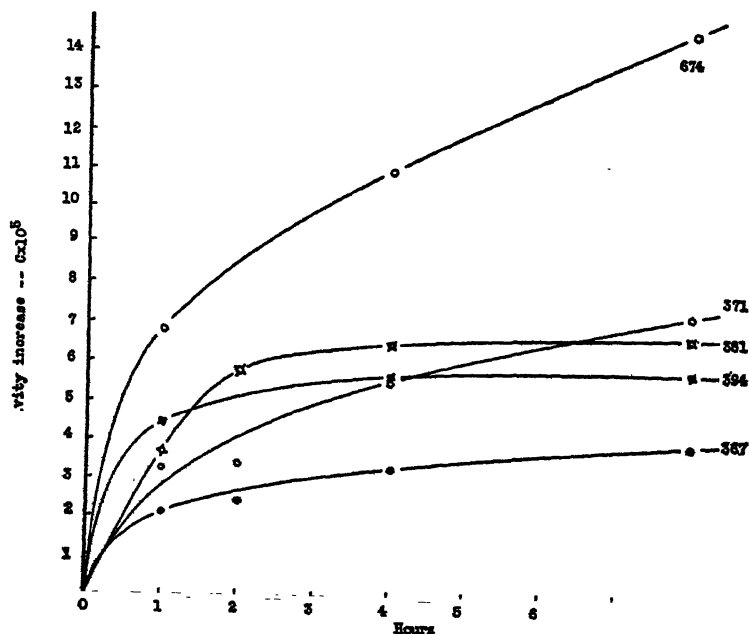
FIG. 2. CONDUCTIVITY INCREASE DUE TO HYDROLYSIS

The hydrolyzability of soil material is largely influenced by the nature of the complex, the amount and nature of the adsorbed base, and the pH. From the point of view of conductivity increases, the following three possibilities present themselves:

(a) In unsaturated soils of low pH the total amount of hydrolyzable metallic ions is small, the complex being nearly saturated with H ions. If such a complex were stable, the amount of hydrolysis and therefore conductivity increase would be negligible, as the H ion is the most actively adsorbed ion. As it happens, however, colloidal ferric silicate complexes at a pH below 5.4 become highly unstable (16), and solubility effects on the iron in the complex become noticeable. Any increases in conductivity in acid soil must therefore

be greatly influenced by the disintegration and solution of the soil complex itself.

(b) As the complex becomes more saturated with bases at higher pH values, it becomes highly stable, and in normal soils the predominant adsorbed bases are divalent Ca and Mg ions. Hydrolysis here would set free cations of importance in plant nutrition. The power of a soil to maintain a steady supply of such hydrolyzed material would depend on the percentage of colloidal matter, i.e., on the degree of weathering.
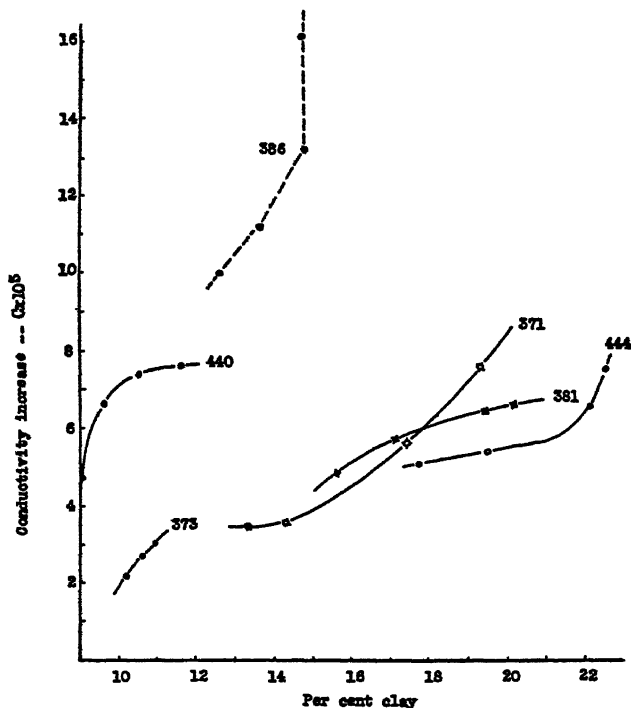
FIG. 3. RELATION BETWEEN DISPERSION AND CONDUCTIVITY INCREASE

(c) In alkaline soils where Na ions predominate, the effect of dispersion and hydrolysis would be enormous due to the hydration of the Na ion. In the presence of a large excess of soluble salts, this would naturally be prevented and under such circumstances it might be found that hardly any increases in conductivity would take place after initial equilibrium is attained. Such a state of affairs would therefore bear no relation whatever to bacterial activity, as was indicated by Sen. In a solonetz, where the Na complex is largely free from soluble sulfates and chlorides, the possibility exists that hydrolysis might be so rapid as almost to be complete in the time required for the establishment of equilibrium of easily soluble material. In such a case there would also be

no conductivity increases over long periods. In soil formation the latter process is known as solodification and can in time give rise to a degraded solonetz saturated with H ions.

CONDUCTIVITY AND FERTILITY

It has been shown that conductivity measurements can be used to determine the amount of readily soluble electrolyte in a soil suspension (1:5) at any moment, as well as the changes in electrolyte content resulting from bacterial

TABLE 4

Hydrolysis of soil due to boiling

SOIL NUMBER	INITIAL CONDUCTIVITY $C \times 10^{-6}$	TIME OF BOILING							
		1 hour		2 hours		4 hours		8 hours	
		$C \times 10^{-6}$ Δ*	Clay	$C \times 10^{-6}$ Δ	Clay	$C \times 10^{-6}$ Δ	Clay	$C \times 10^{-6}$ Δ	Clay
			per cent		*per cent*		*per cent*		*per cent*
404	13.39	16.0	....	20.9	....	27.0	.....	35.1	.....
382	30.3	22.5	....	28.8	....	39.7	.....	57.1	.....
405	31.91	30.9	....	36.8	....	45.9	.....	52.2	.....
406	42.21	18.4	....	23.8	....	35.7	.....	57.9	.....
388	69.00	47.2	....	56.5	....	74.8	.....	94.2	.....
367	80.3	21.9	....	24.8	....	32.5	.....	40.5	.....
361	84.7	21.0	....	41.0	....	47.6	.....	72.2	.....
444	119.8	49.3	17.6	51.7	19.3	63.5	21.9	72.8	22.2
386	134.3	97.1	12.5	109.5	13.5	128.2	14.58	156.2	14.58
364	138.4	34.5	....	35.8	....	48.7	.....	56.01	.....
394	141.5	43.1	....	47.4	....	55.2	.....	58.5	.....
373	164.9	21.2	10.28	20.0	....	26.4	10.6	29.5	10.94
440	185.8	47.6	9.1	64.0	9.6	71.6	10.5	76.1	11.5
371	211.3	32.6	13.2	34.0	14.2	53.7	17.3	73.1	19.0
409	213.7	37.5	....	54.3	....	66.8	.....	81.87	.....
381	255.3	36.4	15.5	54.8	17.0	63.4	20.0	67.0	.....
674	265.0	67.7	....	77.0	....	109.1	.....	146.8	.....
355	291.8	27.7	11.7	39.5	11.7	.....	12.4	49.04	13.0
673	1,073.0	52.2	....	....	....	130.9	.....	186.0	.....

* $\Delta . C \times 10^{-6}$ is difference between conductivity and "initial conductivity."

activity, hydrolysis, and dispersion. To correlate such measurements with crop response is to assume that soil fertility, i.e., the power of a soil to produce crops, is not only primarily a matter of electrolyte content but also one independent of the nature of the electrolyte. It is, however, common knowledge that insofar as crop response can be correlated with soil conditions alone, the main factors are the availability of specific mineral nutrients, which in their turn depend on organic matter content, microbial activity, and physical and moisture conditions.

Proof has further been advanced that conductivity increases, measured in

this way, bear scant relation to the microbial activity of the soil, the factors which advance or retard it, and therefore to the important process of nitrification. They do, however, serve as a rough measure of the hydrolyzability of soil material, i.e., the ability of the soil to split off adsorbed bases whether such bases have a plant nutritional value or not. This can, however, be far more efficiently determined by boiling a soil with water in the absence of CO_2 than by the "7 days' increase" method, and from table 5, where these two determinations are compared, it is obvious that no relation whatever exists between them. Although one would naturally hesitate to correlate crop yields with these values, this has been done here for comparative purposes. Since most of

TABLE 5

Relation between "7 days increase," hydrolysis, and crop yield

SOIL NUMBER	7 DAYS INCREASE $C \times 10^{-6}$	8 HOURS BOILING INCREASE $C \times 10^{-6}$	RELATIVE CROP YIELD	REMARKS
404	27.5	35.0	...	.
364	19.0	36.0	480	Strong N, no P response
367	6.3	40.5	580	N P response, no K
355	14.6	49.0	589	Weak P and K, strong N response
405	43.9	52.2	...	
382	53.0	57.1	...	
406	9.6	57.9	...	
394	50.7	58.5	...	
381	42.3	67.0	685	P and strong K response
361	13.8	72.2	906	Strong N P, no K response
371	9.0	73.1	...	
409	13.0	81.8	...	
388	64.9	94.2	800	Strong K P response
674	94.0	148.2	...	
386	32.9	156.2	...	
673	226.0	186.0	...	

the soils are from the check plots of wheat manurial trials, both their chemical composition and their nutrient deficiencies are known.

In most of them too, climate, drainage condition, organic matter content, and texture are very similar indeed, so that there is some justification for this comparison. It will be noticed from table 5 that crop yields increase with increasing hydrolysis as measured by 8 hours boiling in water, but bear no relation whatever to the 7 days' increase. This brings out the further point that in soils where conditions are essentially the same, and where crop differences are largely due to mineral deficiencies, correlations of the kind obtained by Sen on the Rothamsted plots, stretching over many years, can be expected. Its application in a similar way to soils in general, however, is entirely unwarranted.

Although conductivity measurements are invaluable in the study and mapping of saline soils, their application to soil fertility studies on the one hand, and to the nature of the colloidal complex on the other, is undoubtedly subject to very considerable limitations.

SUMMARY

Conductivity changes in soil suspensions in the absence of atmospheric CO_2 are caused by (a) microörganic activity, (b) changes in degree of dispersion of the solid phase, (c) dissociation of electrolytes, resulting from solution of easily soluble salts and from hydrolysis of the colloidal complex.

Microörganic activity in "7 days' increase" is chiefly aerobic and ceases after a few days of incubation. This contribution to conductivity increase is arbitrary and analogous to shaking up a soil with water containing CO_2. It bears no relation to the true microörganic activity of the soil.

During incubation, pH increases as a result of hydrolysis of adsorbed bases and possibly some solution due to the presence of CO_2, with formation of bicarbonates.

Cataphoresis and surface adsorption are limiting cases of conduction in electrolytes.

The finer sol particles contribute to the conduction of a current, and the degree of dispersion of the soil is therefore an important factor in conductivity determinations.

Hydrolyzability of the soil complex is a function of the nature of the complex, the nature and quantity of adsorbed ions, and the pH.

In physicochemical comparisons of the colloids in different soil types, equivalent quantities of colloid as regards base exchange capacity must be used. The amphoteric nature of soil colloids sets a serious limit to the usefulness of such measurements.

The relationships between fertility, "7 days' increase," and hydrolysis are discussed.

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THE LAWS OF SOIL COLLOIDAL BEHAVIOR: XV. THE DEGRADATION AND THE REGENERATION OF THE SOIL COMPLEX[1]

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When the soil complex reacts with the common, easily displaceable ions the process is reversible and the complex is left intact, retaining its identity, its isoelectric point, and its exchange capacity. When other ions which form very slightly dissociated combinations react with the soil complex the latter changes its character and behavior. Such strongly associated ions become a part of the colloidal ion complex and modify its properties by changing the strength and magnitude of the acidic and basic residues. Thus when phosphate, silicate, or humate ions react with a soil colloid (especially one having a low silica/sesquioxide ratio) there is an increase in the strength of the acidic and a decrease in the basic residue, a lowering of the isoelectric point, and an increase in the cation exchange capacity. Conversely if aluminum or a similar ion is precipitated with a soil colloid (especially one having a high silica/sesquioxide ratio) there is a decrease in the strength of the acidic and an increase in the basic residue, an elevation of the isoelectric point, and a decrease in the cation exchange capacity. These facts were brought out in some of the preceding chapters of this series (3, V, VI), but since then a more detailed study of this phenomenon has been made, the results of which will be here discussed.

THE CHEMICAL NATURE OF THE EXCHANGE COMPLEX

The soil complex consists of weak, mostly insoluble or slightly soluble polyvalent acids and bases. To illustrate the reactions of compounds of such acids and bases we shall again make use of the following scheme:

Assume a tribasic acid $H_I H_{II} H_{III} A$ and a base $BOH_{III} OH_{II} OH_I$. The activities of the three H ions are

$$H_I > H_{II} > H_{III}$$

and that of the OH ions

$$OH_I > OH_{II} > OH_{III}$$

The most simple combinations between the acid and the base would result when equivalent proportions react thus:

$$\text{H}_\text{I}\ \text{H}_\text{II}\ \text{H}_\text{III}\ \text{A} + \text{BOH}_\text{III}\ \text{OH}_\text{II}\ \text{OH}_\text{I} \leftrightarrows \qquad (A)$$

$$\text{H}_\text{II}\ \text{H}_\text{III}\ \text{A} \cdot \text{BOH}_\text{III}\ \text{OH}_\text{II} + \text{H}_2\text{O} \leftrightarrows \qquad (B)$$

$$\text{H}_\text{III}\ \text{A} : \text{BOH}_\text{III} + \text{H}_2\text{O} \leftrightarrows \qquad (C)$$

$$\text{A} : \text{B} + \text{H}_2\text{O} \qquad (D)$$
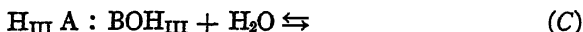
The extent to which the mutual combination or neutralization will take place will depend upon the ionization of the acid, of the base, and of the salt, for although the latter is insoluble it ionizes to some extent and this will prevent a complete "neutralization." The compound will, in other words, remain more or less hydrolyzed. If the product

$$[\text{H}_\text{III}]\ [\text{OH}_\text{III}] < K_w$$

and if the ionization

$$\text{A} : \text{B} \rightarrow \text{A}^- : \text{B}^+$$
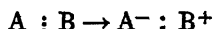
proceeds to a certain slight extent the reaction would stop at (C) and we would have the compound

$$\text{HA} : \text{BOH} \qquad (E)$$

In other than equivalent proportions of acid and base the reaction would be more complex and give rise to compounds of the type

$$\begin{matrix} \text{H}_2\text{A} & \\ & \diagdown \\ & \text{BOH} \qquad (F) \\ & \diagup \\ \text{H}_3\text{A} & \end{matrix}$$

and

$$\begin{matrix} & \text{B(OH)}_2 \\ & \diagup \\ \text{HA} & \\ & \diagdown \\ & \text{B(OH)}_2 \end{matrix} \qquad (G)$$

Furthermore if both the acid and the base are themselves insoluble they might enter in any proportion into the make up of the colloidal micelle, which contains thousands or millions of molecules.

The isoelectric point of the various compounds will be governed by the relative strength of the acid and basic residue. The isoelectric point of compound (F) will be lower than that of compound (E) and will be highest in the case of compound (G). These compounds will react amphoterically: with

bases at pH values above their isoelectric point and with acids below this point. If the isoelectric point is lower than pH 7.0, that is, if the acid residue is the strongest, as is usually the case in soil colloids, the complex will adsorb and exchange cations from neutral salt solutions. The cation exchange capacity will obviously be greatest in (F) and smallest in (G) (per gram colloid). The ultimate pH will be lowest in (F) and highest in (G).

<div align="center">EXCHANGE REACTIONS</div>

We shall now see how we might expect these compounds to react with different electrolytes. These amphoteric compounds are themselves salts whose residual H and OH ions are attached to spatially separate groups whose union depends upon the pH of the medium. Their behavior will therefore be different from the behavior of ampholytes whose acidic and basic characters reside in a single group such as the amphoteric hydroxides. It is obvious that the union, i.e., the number of bonds, between A and B will be at a maximum in the absence of stronger acids and bases, that is, at the ultimate pH or near the isoelectric point. If a strong base is added to a compound of type (E) the H ion will first be displaced thus:

$$NaOH + HA : BOH \rightarrow NaA : BOH + H_2O$$
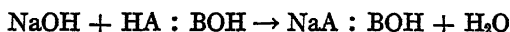
But the reaction will not stop here, for sooner or later as the pH is increased the compound will undergo hydrolytic cleavage, resulting in the following change:

$$NaOH + NaA : BOH \rightarrow Na_2A \cdot B(OH)_2$$

and finally at a sufficiently high alkalinity:

$$NaOH + Na_2A \cdot B(OH)_2 \rightarrow Na_3A + B(OH)_3$$

This hydrolytic reaction, which undoubtedly takes place in the soil, is very important, for it shows that the amount of base which a soil will bind is greater than the number of equivalents of displaceable hydrogen actually present in the unsaturated soil. It has previously been shown $(3, V)$ that the cation exchange capacity of a soil colloid can be built up permanently by treating the material with strong alkali. The process undoubtedly consists in a hydrolytic cleavage of the amphoteric complex, that is, in an unlocking of the bonds between the acidoid and basoid groups. How much of the hydrogen displaceable at pH 7.0 by the neutral salt treatment actually exists as such in the unsaturated soil is difficult to say. It is certain that the displaceable hydrogen does not correspond to the amounts of adsorbed bases at higher pH values.

A similar hydrolytic cleavage results, of course, when an amphoteric compound of this type is treated with strong acids.

<div align="center">THE ISOELECTRIC DECOMPOSITION OR DEGRADATION OF THE COMPLEX</div>

The presence of strong acids or bases leads to a suppression of the ionization of the corresponding group in the amphoteric complex whereas the ionization

of the opposite group is greatly increased. *Now since the ionized (charged) group is more dispersible and soluble than the unionized (uncharged) group it follows that the colloidal complex will, through a hydrolytic cleavage, lose some of the former and become enriched in the latter group.* At pH values above the isoelectric point the acid group will be ionized and will to some extent pass into solution. If the soluble products are removed by leaching, as under natural conditions in the soil, the process is irreversible. The complex will become richer in the basic group, and this will elevate the isoelectric point until it approaches the prevailing pH when the complex will again be stable. Instead of the original compound HA : BOH we shall end up with a more basic one, as for example

$$HA \Big\langle {}^{B(OH)_2}_{B(OH)_2}$$
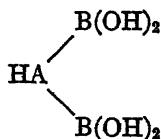
At pH values below the isoelectric point the basic group will be ionized and split off, leaving a complex which possesses a stronger acid residue, a lower isoelectric point, and a greater stability at the lowered pH as indicated by the following formula:

$$ {}^{H_2A}_{H_2A} \Big\rangle BOH $$
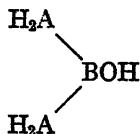
This process, by which the activated, ionized group continually tends to split off from the complex was laid as a basis for the theory of isoelectric weathering (3, IX) and is represented in nature by podzolization as one extreme and laterization as the other.

ISOELECTRIC SYNTHESIS OR REGENERATION OF THE COMPLEX

A simple rule of the mass law is that when two ions which together constitute an insoluble compound are mixed, then that compound will be formed. This rule might be applied to colloids as follows:

When an ion which, in combination with a colloid, forms an isoelectric complex is added to the colloid then that complex will be formed in preference to all other combinations, and the displaceable ions already in combination with the colloid will be effectually displaced. An ion of this type can only be displaced by other ions of a similar type or by hydrolytic action at pH values far above or below the isoelectric point. If the colloid is originally an amphoteric colloid then its isoelectric pH will be deflected: upward if the ion be a cation and downward if it be an anion.

The isoelectric complex is least ionized and represents, therefore, the most stable condition. The complex will, therefore, strive to assume this condition. This can be accomplished not only by the loss through a hydrolytic cleavage of

the ionized active group as already outlined but also by the neutralization of the charge through the association of a non-dissociable ion.   In contradistinction to the term "isoelectric decomposition" this process might be termed "isoelectric synthesis."   It constitutes the basis for the regeneration of soils.

Before we present the experimental work dealing with this problem we shall devote a few additional lines to a theoretical treatment of the reactions envolved.

Suppose the pH x of the medium be such that a compound of the acid H_3A and base $B(OH)_3$ would be isoelectric in equivalent proportions thus

$$HA : BOH$$

But assume now that the actual composition of the compound be

$$HA \Big\langle {\overset{\textstyle B(OH)_2}{\underset{\textstyle B(OH)_2}{}}}$$
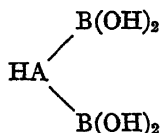
the isoelectric point of which will be higher than x.   The compound will therefor exist in a partly combined condition with acid anions, e.g., Cl, thus:

$$HA \Big\langle {\overset{\textstyle BOHCl}{\underset{\textstyle BOHCl}{}}}$$
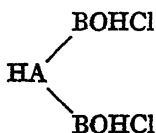
The compound would be positively charged through the dissociation of the Cl ions, but this charge would be neutralized and the compound rendered isoelectric in the presence of sufficient quantity of a weak acid of the type H_3A, which forms a nondissociated compound with B according to the equation

$$HA \Big\langle {\overset{\textstyle BOHCl}{\underset{\textstyle BOHCl}{}}} \quad + H_3A \rightarrow 2\ HA{:}BOH + 2\ HCl$$
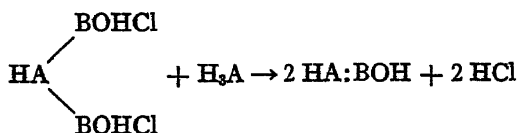
If we assume the composition of the original compound to be

$$\overset{\textstyle H_2A}{\underset{\textstyle H_2A}{}} \Big\rangle BOH$$
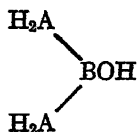
then its isoelectric point will be lower than x and the compound will exist in a partly saturated condition with metal cations, e.g., Na, thus

$$\overset{\textstyle NaHA}{\underset{\textstyle NaHA}{}} \Big\rangle BOH$$
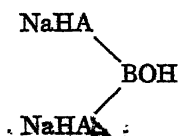
This compound, which will be negatively charged, will be rendered isoelectric in the presence of a sufficient quantity of a weak base of the type $B(OH)_3$ according to the following equation:

$$\begin{array}{c} NaHA \\ \diagdown \\ \quad\quad BOH + B(OH)_3 \rightarrow 2\ HA : BOH + 2\ NaOH \\ \diagup \\ NaHA \end{array}$$
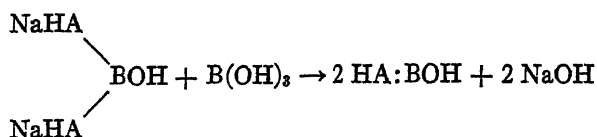
In both cases we obtain a compound of the type HA : BOH, which on assumption is isoelectric at pH x. In the first case the isoelectric point is lowered and the cation exchange capacity is increased, whereas in the second case the effect is the opposite. The power of the complex to bind anions is decreased in the first case and increased in the second.

THE ALUMINATED SOIL COMPLEX

The last type of reaction takes place when a soil is isoelectrically precipitated with an aluminum salt. In table 134 the cation exchange capacity of the original and that of the aluminated Sharkey and Sassafras soil colloids are compared. The isoelectric point was determined exactly as in the case of bentonite as shown in table 58 A (3, IV). With 1.5 millimol $AlCl_3$ per gram Na-saturated colloid the Sharkey was isoelectric at pH 6.0 and the Sassafras at pH 6.8.

The isoelectric precipitation in bulk was then brought about by mixing 1 liter containing 1 gm. Na-saturated colloid plus the required amount of NaOH with 1 liter containing 1.5 millimol $AlCl_3$. The precipitate was then placed on the filter and the cation exchange capacity determined by the neutral Ba-acetate method as in the case of the original colloid. The isoelectric point of the original Sassafras colloid in HCl was about pH 4.3. In the case of the Sharkey colloid this value cannot be determined cataphoretically by our cell because of the evolution of gas at the electrodes at the low pH required, but judging by the pH of exchange neutrality of this colloid in a NaCl solution the isoelectric point would be about pH 3.0.

The 1.5 millimol $Al(OH)_3$ tied up and inactivated more than 60 per cent of the exchange valences active at a pH of 7.0. The earlier experiments with bentonite yielded the same results. The considerably weaker base $Fe(OH)_3$ produced a much smaller effect. It is obvious that a still stronger base which yet forms a practically nondissociated compound with the acid group of the colloid, and would therefore not be displaceable by the neutral salt cations, would still further reduce the cation exchange capacity. Thus Anderson and Byers (1) found that ferrous hydroxide in certain cases entirely suppressed the ammonia adsorption by soil colloids.

The following equation will serve to illustrate why the isoelectric point is raised and the cation exchange capacity is reduced by the reaction:

$$
\begin{array}{c}
\text{H} \\
| \\
\text{A—Na} \quad \text{OH} \\
\diagup \qquad \diagdown \\
\text{OH—B} \qquad + \qquad \text{Al—OH} \\
\diagdown \qquad \diagup \\
\text{A—Na} \quad \text{OH} \\
| \\
\text{H}
\end{array}
\rightarrow
\begin{array}{c}
\text{H} \\
| \\
\text{A} \\
\diagup \qquad \diagdown \\
\text{OH—B} \qquad \text{Al—OH} + 2\,\text{NaOH} \\
\diagdown \qquad \diagup \\
\text{A} \\
| \\
\text{H}
\end{array}
$$
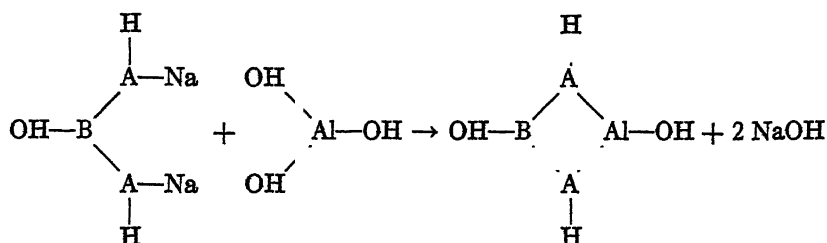
A and B represent the acid and basic groups in the original colloid. It will be noted that the basic residue has been increased and that the acid residue has been decreased by the reaction. Both of the aluminated colloids were isoelectric below pH 7.0 and retain, therefore, some power to adsorb and exchange cations at this point.

It is interesting to speculate about the action of the fairly strong base ferrous hydroxide in a soil under reducing conditions, below the ground water level. The ferrous ion is here formed by the reduction of the very weak base, ferric

TABLE 134

The cation exchange capacity of the original and the aluminated Sharkey and Sassafras soil colloids

Milliequivalents per gram

COLLOID	ISOELECTRIC POINT		EXCHANGE CAPACITY		DECREASE IN EXCHANGE CAPACITY
	Original	Aluminated	Original	Aluminated	
	pH	*pH*	*m.e.*	*m.e.*	*per cent*
Sharkey......................	3.0 ?	6.0	0.783	0.267	66.0
Sassafras....................	4.3	6.8	0.326	0.125	60.7

hydroxide. A weak base changes to a strong base whose cation powerfully displaces the exchangeable cations of the common still stronger bases. This fact readily explains the high pH common in such soils even in the colder regions of heavy rainfall. If the displaced bases are leached out and if the soil is then drained, the iron will be oxidized and largely hydrolyzed into free $Fe(OH)_3$ thus leaving an unsaturated acid residue in the soil complex. Such and other conclusions may be drawn, but, since the problem is now being investigated, we shall defer any further discussion.

There is, however, another point of interest in connection with the last experiment. This concerns the application of aluminum sulfate to suppress the alkalinity of solonetz soils. This has been tried by several workers (2), who found that the pH of the soil was temporarily lowered but climbed rather rapidly back to its original value. The original lowering of the pH is, of course, due to the sulfuric acid formed by hydrolysis. Some of the acid, however, remains in combination with the alumina even at high pH values. The later reversion of the pH is explained by the last equation. Some of the exchange-

able cations are displaced by Al, and the cation exchange capacity of the soil complex is permanently reduced. The acidic character of the complex has been weakened, its isoelectric point has been raised, and its power to bind bases is thereby partly suppressed. The method is obviously at fault.

THE SILICATED AND PHOSPHATED SOIL COMPLEX

If the OH ions in the basic residue of the amphoteric colloidal complex be displaced by the polyvalent anions of a weak acid which form a strongly associated compound with the basic group, there will result a new complex having a stronger acid residue, a weaker basic residue, a lower isoelectric point, and a higher cation exchange capacity.

Tables 135 and 136 show the effects of silicic and phosphoric acids upon the cation exchange capacity of the Sharkey, the Sassafras, and the Nipe soil colloids. Since the isoelectric point could not be determined in all cases, the colloids were all precipitated at the same pH. Although the strongly associat-

TABLE 135

The cation exchange capacity of the original and the silicated Sharkey, Sassafras, and Nipe soil colloids

Milliequivalents per gram

COLLOID	EXCHANGE CAPACITY		SiO₂ ADSORBED	INCREASE IN EXCHANGE CAPACITY	
	Original	Silicated			
	m.e.	m.e.	mgm.	m.e.	per cent
Sharkey...................	0.783	0.787	1.5	0.004	0.5
Sassafras.................	0.326	0.355	3.5	0.029	8.9
Nipe.....................	0.126	0.247	18.5	0.121	96.0

ing anions will displace OH ions far above the isoelectric point of the colloids, the adsorption is greater at low pH where the OH ions in solution offer but little competition. Furthermore, a complete flocculation is obtained only near the isoelectric point. A pH of 3.2 was found suitable. This was far below the isoelectric point of the original Sassafras (about 4.3) and Nipe (about 6.0) and near that of the Sharkey.

The precipitation was carried out in the following manner:

One liter of a solution containing 7.5 millimols sodium silicate, for the silicate series, and 7.5 millimols disodium phosphate for the phosphate series, was rapidly mixed with 1 liter containing 1 gm. suspended colloid together with sufficient HCl to give a final pH of 3.2. The following day the clear, supernatant liquid was decanted, the precipitates were air dried, and the cation exchange capacity was determined as before stated. A second phosphate series was prepared with the same proportions but with a dilution of only one-tenth that of the first. This series is designated as the concentrated series.

The results show that the colloid with the highest isoelectric point, i.e., the

Nipe, adsorbs the greatest quantity of silicate and phosphate ions and exhibits the greatest increase in exchange capacity. The Sharkey colloid adsorbed so little SiO_2 and P_2O_5 from the dilute solutions that the small, yet fairly proportional, increases in the exchange capacities recorded must be looked upon as accidental results rather than as true values, since duplicate samples generally show greater differences.

In the concentrated series the Sharkey showed the highest increase in a proportionally higher phosphate adsorption whereas the increase in exchange capacity was small compared to that of the other colloids. We believe this difference to be real and to be due to a displacement of the silicate ion by the phosphate, which was proved by an analysis of the supernatant liquid.

TABLE 136

The cation exchange capacity of the original and the phosphated Sharkey, Sassafras, and Nipe soil colloids

Milliequivalents per gram

COLLOID	EXCHANGE CAPACITY		P_2O_5 ADSORBED	INCREASE IN EXCHANGE CAPACITY	
	Original	Phosphated			
	m.e.	*m.e.*	*mgm.*	*m.e.*	*per cent*
Sharkey........................	0.783	0.789	1.0	0.006	0.8
Sassafras......................	0.326	0.399	2.9	0.073	22.4
Nipe...........................	0.126	0.236	16.8	0.110	87.3
Concentrated series					
Sharkey........................		0.845	14.0	0.062	7.9
Sassafras......................		0.503	16.0	0.177	54.5
Nipe...........................		0.332	18.0	0.206	165.0

The increase in the cation exchange capacity and the lowering of the isoelectric points may be illustrated by the equation:

$$
\begin{array}{c}
\mathrm{OH} \\
|\\
\mathrm{B-OH} \quad \mathrm{H} \qquad \mathrm{H} \\
/ \qquad \backslash / \\
\mathrm{H-A} \qquad + \quad \mathrm{SiO_4} \\
\backslash \qquad / \backslash \\
\mathrm{B-OH} \quad \mathrm{H} \qquad \mathrm{H} \\
| \\
\mathrm{OH}
\end{array}
\qquad
\begin{array}{c}
\mathrm{OH} \\
|\\
\mathrm{B} \qquad \mathrm{H} \\
/ \backslash \quad / \\
\mathrm{H-A} \quad \mathrm{SiO_4} \quad + 2\,\mathrm{H_2O} \\
\backslash \quad / \backslash \\
\mathrm{B} \qquad \mathrm{H} \\
| \\
\mathrm{OH}
\end{array}
$$
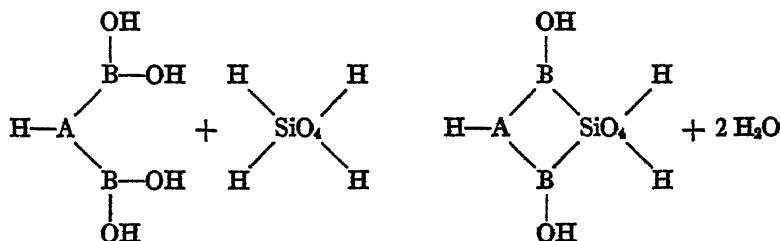
This synthetic upbuild or regeneration of the soil complex must take place in the soil under certain favorable conditions. Thus the acidic complex in the A_2 horizon of a podzol will, at the cessation of the podzolizing conditions (the destruction of the raw humus covering), combine with and fix the weak bases previously kept mobile in the ionized condition. Through the weathering of the

minerals, through the action of burrowing animals, and through the decomposition of plant residue sufficient bases will finally be available for the synthetic regeneration of a more basic complex of the type met with in the brown soils. Such a complex will possess two advantages over the originally very acidic complex: (a) It will, by virtue of its stronger basic residue, more firmly fix the valuable PO_4 anion, and (b) it will require a smaller quantity of exchangeable cations (bases) to maintain a favorable pH.

The extremely basic complex in lateritic soils will, under certain conditions, combine with weak acids such as silicic and humic acids especially when these are present in a fairly acid soil solution. The complex thus built up would possess a stronger acid residue, the advantages of which would be: (a) a moderate power to bind cations, a power which is almost lacking in the laterites, and (b) a less firm fixation of the PO_4 anion, a fixation which is often too firm in the laterites.

The chances of a regeneration of the lateritic complex are probably more remote than in the case of the podzolic complex. The laterites are very deep, the source of silicic acid is largely exhausted, and humic acids do not long endure in lateritic regions. Nevertheless silicic acid is always pumped up by plants, and a certain change in the climate, drainage, and vegetation might very well lead to a regeneration of the lateritic soil complex.

SUMMARY

The amphoteric soil complex which represents partly hydrolyzed salts of polyvalent weak acids and bases is subject to the following destructive and constructive changes:

At low and at high pH the basic and the acidic groups, respectively, become ionized and are split off by hydrolytic cleavage and, since the ionized condition represents the most soluble, these groups may be permanently lost by the soil. This process leads to a degradation of the soil.

Under other conditions, favorable for the reaction, the degraded complex will unite with acidic or basic groups with which an isoelectric compound may be formed. Conditions permitting this synthetic upbuild will lead to a regeneration of the soil. A study of the aluminated, the silicated, and the phosphated soil colloids shows the conditions and nature of such reactions.

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EFFECT OF SUNLIGHT ON THE NITRIFICATION OF AMMONIUM SALTS IN SOILS

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In two recent publications, Dhar, et al. (2, 6) claim that the process of nitrification in the soil, especially in the tropics, is more a photochemical than a bacterial process. Other claims have recently been made that nitrification by simple physico-chemical action takes place in soils (1) and that photochemical nitrification occurs in sea water (7). The claim that nitrification is more photochemical than bacterial is very interesting to the soil investigator, since up to the present time nitrification has been generally accepted as a process carried out almost entirely by the action of specific microörganisms requiring certain favorable conditions in order to support their activity. A vast amount of experimental nitrification work has been successfully done in which the cultures received no sunlight whatever.

In a previous publication (4), evidence was presented to show that the low nitrification capacity of some Texas soils was due either to insufficient numbers of nitrifying organisms or to lack of calcium carbonate present in the soil, or to both. These experiments were all carried out in darkness in electrically heated incubators. The photochemical aspects of the problem, as claimed by Dhar et al., presented a new phase which required investigation. Accordingly, work was carried out at College Station, the results of which are presented in this paper.

PLAN AND METHOD OF WORK

The nitrification tests were carried out on six samples of soil from widely separated parts of Texas. Some information regarding these soils is given in table 1. One portion of the original soil was used without treatment, another was inoculated to increase the number of the nitrifying organisms originally present, a third portion was sterilized, and a fourth was sterilized and inoculated. Each was used both with and without ammonium salts. In each case one set was exposed to direct sunshine, while the other set was placed beside it, but protected from the light. The plan of the experiment is shown in tables 2 and 3. Portions of 200 gm. of air-dry soil were mixed with 10 cc. of a solution of ammonium salt shown by analysis to contain 0.10 gm. nitrogen, equal to 500 p.p.m. nitrogen in the dry soil, and sufficient water to equal 50 per cent of the water capacity of the soil. A similar preparation was made without any addition of ammonium salt. After a thorough mixing, the soil was transferred to

85

300-cc. tall Pyrex beakers, packed by striking the beaker on the palm of the hand, weighed, and placed outdoors during the day. The beakers exposed to the sunlight were covered with window glass in order to keep the dust out. Corresponding cultures were kept in the dark, being placed at the side of the exposed cultures but covered with galvanized iron cans. The ammonium salts used were reagents of tested purity.

For the preparation of the inoculating liquid which was added to some of the cultures, 20 gm. of soil from a soil culture was placed in a mortar with a few cubic centimeters of water and ground thoroughly to a thin paste. More water was added, a little at a time, with grinding, the mixture transferred to a 500-cc. volumetric flask, and made up to volume. The soil suspension was thoroughly shaken immediately before the aliquot of 10 cc. used for each culture was taken out.

The soils were sterilized by heating 200-gm. portions in 300-cc. tall Pyrex beakers, covered with watch glasses, in an electric oven for 2 hours at 140°-

TABLE 1

Description of soils

SOIL NUMBER	TYPE NAME	COUNTY	pH	DEPTH	BASICITY	NITROUS N	NITRIC N
				inches	*per cent*	*p.p.m.*	*p.p.m.*
9691	Houston black clay, subsoil	Wise	7.56	8 to 14	18.00	0	1.5
23094	Houston black clay (probably)	Dallas	7.20	0 to 7	4.91	0	9.4
32922	Victoria fine sandy loam (probably)	Karnes	7.67	Surface	5.01	0.3	16.3
36370	Shamrock very fine sandy loam	Wheeler	7.91	0 to 2	4.26	0.2	1.5
36473	Clareville clay loam	Bee	7.84	7 to 20	2.00	0.1	1.8
36498	Houston black clay	Bell	7.51	Surface	10.00	0.03	29.4

150°C. This procedure was found by previous tests to destroy the nitrifying organisms. The spatulas and porcelain dishes used for each culture were also sterilized.

The temperatures were taken daily, except Sunday, between 3:00 and 4:00 p.m. by means of a thermometer in one of the cultures that was exposed in the sunlight, and from another thermometer in one of those that were kept in the dark.

The cultures placed in the sun lost water more rapidly than did those left in the dark, but sufficient water to restore the loss in weight was added daily to all cultures. In order to expose the soil to the light as completely as possible, all cultures were thoroughly stirred with spatulas daily before the water was added.

After 28 days, each culture was weighed, emptied into a dish, mixed thoroughly, and an aliquot of one-tenth taken for the determination of nitrates and nitrites. The determinations of nitrates were made by the phenol-disulfonic acid method, and those for nitrites by the alpha-naphthylamine method (3).

TABLE 2

Net amounts of ammonia nitrogen changed to nitrates and nitrites in sunlight and in the dark

LAB. NUMBER OF SOIL	CONDITION OF SOIL	IN PARTS PER MILLION OF DRY SOIL						IN PERCENTAGE OF ADDED AMMONIA					
		(NH₄)₂SO₄		NH₄Cl		(NH₄)₂HPO₄		(NH₄)₂SO₄		NH₄Cl		(NH₄)₂HPO₄	
		Sunlight	Dark	Sunlight	Dark	Sunlight	Dark	Sunlight	Dark	Sunlight	Dark	Sunlight	Dark
9691	Unsterilized	0	98	0	0	0	109	0	20	0	0	0	22
9691	Unsterilized + inoculated liquid	0	217	0	31	1	475	0	43	0	6	0	95
9691	Sterilized	0	8	0	0	0	0	0	2	0	0	0	0
9691	Sterilized + inoculated liquid	0	86	0	0	8	119	0	17	0	0	2	24
23094	Unsterilized	0	278	0	133	19	358	0	56	0	27	4	72
23094	Unsterilized + inoculated liquid	0	411	0	228	54	474	0	82	0	46	11	95
23094	Sterilized	0	0	0	0	0	0	0	0	0	0	0	0
23094	Sterilized + inoculated liquid	11	45	0	134	24	173	2	9	0	27	5	35
32922	Unsterilized	0	419	3	235	14	484	0	84	1	47	3	97
32922	Unsterilized + inoculated liquid	0	434	4	324	56	477	0	87	1	65	11	95
32922	Sterilized	1	1	0	9	0	2	0	0	0	2	0	0
32922	Sterilized + inoculated liquid	0	9	0	270	18	227	0	2	0	54	4	45
36370	Unsterilized	0	394	0	155	358	515	0	79	0	31	72	103
36370	Unsterilized + inoculated liquid	0	411	0	409	336	527	0	82	0	82	67	105
36370	Sterilized	0	125	0	0	0	4	0	25	0	0	0	1
36370	Sterilized + inoculated liquid	0	90	2	33	19	29	0	18	0	7	4	6
36473	Unsterilized	0	440	0	160	187	405	0	88	0	32	37	81
36473	Unsterilized + inoculated liquid	0	452	0	387	247	471	0	90	0	77	49	94
36473	Sterilized	0	0	0	0	0	1	0	0	0	0	0	0
36473	Sterilized + inoculated liquid	0	231	0	0	5	3	0	46	0	0	1	1
36498	Unsterilized	124	476	0	163	15	495	25	95	0	33	3	99
36498	Unsterilized + inoculated liquid	181	495	0	294	0	515	36	99	0	59	0	103
36498	Sterilized	1	10	0	23	0	1	0	2	0	5	0	0
36498	Sterilized + inoculated liquid	32	220	0	239	6	76	6	44	0	48	1	15
	Average..........	15	223	0.4	134	57	248	3	45	0.08	27	11	50

To avoid any interference of chloride with the determination of nitrates in the cultures which had received ammonium chloride, the chloride was precipitated with silver sulfate. The aliquot of the filtrate was evaporated; the residue after cooling was saturated with 5 cc. of 0.10 per cent silver sulfate solution;

TABLE 3

Effect of sunlight on the nitrification of ammonium sulfate and of soil nitrogen

(Nitrogen in parts per million)

LAB. NUMBER	CONDITION OF THE SOIL	WITH (NH₄)₂SO₄				WITHOUT (NH₄)₂SO₄				SUNLIGHT	CLOUDINESS	AVERAGE TEMPERATURES	
		Sunlight		Dark		Sunlight		Dark					
		Nitric N	Nitrous N	Nitric N	Nitrous N	Nitric N	Nitrous N	Nitric N	Nitrous N			Sunlight	Dark
										hours	*hours*	*°C.*	*°C.*
9691	Unsterilized	1	0	90	80	1	0	25	47	143	20	42.6	39.3
9691	Unsterilized + inoculated liquid	2	0	237	102	2	0	90	32				
9691	Sterilized	0	0	0	0	0	0	0	0				
9691	Sterilized + inoculated liquid	1	0	19	144	1	0	11	66				
23094	Unsterilized	25	16	341	0	28	20	63	0	148	15	42.9	39.7
23094	Unsterilized + inoculated liquid	16	7	479	0	22	11	67	0				
23094	Sterilized	0	0	0	0	0	0	0	0				
23094	Sterilized + inoculated liquid	10	0	0	72	0	0	0	27				
32922	Unsterilized	17	7	475	0	25	12	53	0	164	6	43.2	40.4
32922	Unsterilized + inoculated liquid	12	5	485	0	15	8	51	0				
32922	Sterilized	0	0	0	0	0	0	0	0				
32922	Sterilized + inoculated liquid	1	0	6	10	0	0	3	4				
36370	Unsterilized	50	10	500	1	122	3	107	0	164	6	43.2	40.4
36370	Unsterilized + inoculated liquid	9	0	430	88	38	3	107	0				
36370	Sterilized	0	0	30	95	0	0	0	0				
36370	Sterilized + inoculated liquid	1	0	36	136	1	0	18	64				
36473	Unsterilized	20	1	475	0	31	7	35	0	148	15	42.9	39.7
36473	Unsterilized + inoculated liquid	4	0	487	0	12	4	35	0				
36473	Sterilized	0	0	0	0	0	0	0	0				
36473	Sterilized + inoculated liquid	1	0	60	256	1	0	76	9				
36498	Unsterilized	171	31	533	0	38	0	37	0	130	30	41.6	36.4
36498	Unsterilized + inoculated liquid	239	23	538	0	38	3	33	0				
36498	Sterilized	3	0	5	7	2	0	2	0				
36498	Sterilized + inoculated liquid	11	34	83	210	6	7	71	2				

this was evaporated to dryness; phenol-disulphonic acid was added; and the work was completed as usual.

EFFECT OF SUNLIGHT ON OXIDATION OF AMMONIA

The cultures exposed to light in the summer and early fall received from 130 to 164 hours of bright Texas sunlight, the remainder of the period of exposure

being cloudy. Details of the work are given in tables which will be discussed later. A summary of the results relating to the production of nitrates and nitrites (combined) from the ammonium salts added, as affected by sunlight is given in table 2. In order to measure the oxidation of ammonia, the nitrogen oxidized in the soil alone was subtracted from the nitrogen oxidized in the

TABLE 4

Effect of sunlight on the nitrification of ammonium chloride and of soil nitrogen

(Nitrogen in parts per million)

LAB. NUMBER	CONDITION OF THE SOIL	WITH NH₄Cl				WITHOUT NH₄Cl				SUNLIGHT	CLOUDINESS	AVERAGE TEMPERATURES	
		Sunlight		Dark		Sunlight		Dark				Sunlight	Dark
		Nitric N	Nitrous N	Nitric N	Nitrous N	Nitric N	Nitrous N	Nitric N	Nitrous N				
										hours	*hours*	*°C.*	*°C.*
9691	Unsterilized	2	5	12	43	2	6	24	35	153	15	39.6	38.5
9691	Unsterilized + inoculated liquid	16	2	128	10	18	8	107	0				
9691	Sterilized	0	0	0	0	0	0	0	0				
9691	Sterilized + inoculated liquid	1	0	14	88	2	5	112	25				
23094	Unsterilized	39	12	201	3	44	13	71	0	149	22	40.8	39.4
23094	Unsterilized + inoculated liquid	38	2	303	0	49	7	75	0				
23094	Sterilized	0	0	0	0	0	0	0	0				
23094	Sterilized + inoculated liquid	0	3	251	6	0	1	123	0				
32922	Unsterilized	61	3	297	1	63	2	63	0	149	22	40.8	39.4
32922	Unsterilzed + inoculated liquid	52	2	385	0	45	6	61	0				
32922	Sterilized	4	0	4	6	0	0	1	0				
32922	Sterilized + inoculated liquid	6	0	355	0	8	0	85	0				
36370	Unsterilized	39	13	268	2	122	1	115	0	153	15	39.6	38.5
36370	Unsterilized + inoculated liquid	35	1	525	1	117	8	117	0				
36370	Sterilized	0	0	0	0	0	0	0	0				
36370	Sterilized + inoculated liquid	0	0	105	90	4	5	152	10				
36473	Unsterilized	2	0	190	3	11	4	33	0	153	15	39.6	38.5
36473	Unsterilized + inoculated liquid	1	0	420	0	7	3	33	0				
36473	Sterilized	0	0	0	0	0	0	0	0				
36473	Sterilized + inoculated liquid	0	4	5	37	2	0	16	76				
36498	Unsterilized	13	18	236	0	36	32	83	0	149	22	40.8	39.4
36498	Unsterilized + inoculated liquid	14	8	381	0	29	25	87	0				
36498	Sterilized	0	0	4	29	6	0	5	5				
36498	Sterilized + inoculated liquid	2	0	381	1	5	0	143	0				

corresponding cultures which received the ammonium salts. On an average, the ammonia in the sulfate was oxidized to the extent of 15 p.p.m. in the sunshine and 223 in the dark; in the chloride, 0.4 in the sunlight and 134 in the dark; in the phosphate, 57 in the sunshine and 248 in the dark. The average percentage of the ammonia oxidized was, for the sulfate, 3 in the sunlight and

45 in the dark; for the chloride, 0.08 in the sunlight and 27 in the dark; for the phosphate, 11 in the sunlight and 50 in the dark. The average for all cultures is 24 p.p.m. of soil, oxidized in the sunshine, compared with 202 p.p.m. in the dark, or 5 per cent of the ammonia oxidized in the sunshine compared with 41 per cent in the dark. These averages include all cultures, both sterilized and

TABLE 5

Effect of sunlight on the nitrification of ammonium phosphate and of soil nitrogen

(Nitrogen in parts per million)

LAB. NUMBER	CONDITION OF THE SOIL	WITH (NH4)2HPO4				WITHOUT (NH4)2HPO4				SUNLIGHT	CLOUDINESS	AVERAGE TEMPERATURE	
		Sunlight		Dark		Sunlight		Dark				Sunlight	Dark
		Nitric N	Nitrous N	Nitric N	Nitrous N	Nitric N	Nitrous N	Nitric N	Nitrous N				
										hours	hours	°C.	°C.
9691	Unsterilized	6	14	168	73	4	16	70	62	155	21	39.5	38.1
9691	Unsterilized + inoculated liquid	41	7	750	0	33	14	250	0				
9691	Sterilized	0	0	0	0	0	0	0	0				
9691	Sterilized + inoculated liquid	3	9	215	54	1	3	148	2				
23094	Unsterilized	44	10	421	0	27	8	63	0	150	20	39.5	38.3
23094	Unsterilized + inoculated liquid	98	2	541	0	41	5	77	0				
23094	Sterilized	0	0	0	0	0	0	0	0				
23094	Sterilized + inoculated liquid	22	7	266	5	0	4	93	5				
32922	Unsterilized	57	3	535	0	43	3	51	0	150	20	39.5	38.3
32922	Unsterilized + inoculated liquid	100	3	535	0	43	4	58	0				
32922	Sterilized	5	0	7	0	5	0	5	0				
32922	Sterilized + inoculated liquid	28	10	297	5	13	7	71	4				
36370	Unsterilized	460	8	612	0	110	0	97	0	155	21	39.5	38.1
36370	Unsterilized + inoculated liquid	450	8	625	0	122	0	98	0				
36370	Sterilized	0	0	2	6	0	0	4	0				
36370	Sterilized + inoculated liquid	132	38	90	115	128	23	176	0				
36473	Unsterilized	210	17	440	0	40	0	35	0	155	21	39.5	38.1
36473	Unsterilized + inoculated liquid	295	0	520	0	48	0	36	13				
36473	Sterilized	0	0	0	0	0	0	0	0				
36473	Sterilized + inoculated liquid	5	3	88	1	2	1	44	42				
36498	Unsterilized	28	15	571	0	31	24	76	0	150	20	39.5	38.3
36498	Unsterilized + inoculated liquid	53	7	594	0	34	9	81	0				
36498	Sterilized	6	0	9	0	8	0	8	0				
36498	Sterilized + inoculated liquid	8	0	103	51	2	0	61	46				

unsterilized. The differences would be greater if the sterilized cultures were omitted. In not a single one of the 72 comparisons between sunlight and no sunlight given in table 2, on six soils, with four treatments and with three salts of ammonia, is there any indication of any oxidation of ammonia due to sunlight. In many cases, there is no oxidation at all in the cultures exposed to

sunlight. Where oxidation occurs, it is invariably greater in the cultures kept in the dark than in those in the sunshine. The light is therefore detrimental to nitrification and not beneficial.

Similar results are, for the most part, to be observed in the results with the cultures of the original soils to which no ammonium salts were added (tables 3, 4, and 5). In 48 of these cultures more nitrification is found in those kept in the dark than in those exposed to sunlight. In 18 there was no difference. There are, however, 7 cultures of the unsterilized soil exposed to sunshine in which slightly more nitrates are found than in the corresponding cultures kept in the dark. This difference ranges from 8 to 18 p.p.m. in five of the six unsterilized cultures of soil 36370 (tables 3, 4, and 5), and from 1 to 8 p.p.m. in two cultures of soil 36498 in table 3. Most of the sterilized cultures of the same two soils and the sterilized cultures which were uninoculated with bacteria nitrify more in the dark than in the sunshine, and none of them nitrify more in the sunshine. The apparent slight photonitrification may be partly due to analytical errors but it is more probably due to irregularities in bacterial action; otherwise, it should be observed in the sterilized cultures of the same soils just mentioned. The possibility of a slight photochemical nitrification is not excluded by this work, but the possible amount is so slight in comparison with the bacterial action that it is practically of no significance.

EFFECT OF KIND OF AMMONIUM SALT USED ON THE OXIDATION

Dhar et al. (2) claimed that with 700 hours sunshine, 89 per cent of the nitrogen in ammonium phosphate, 80 per cent of that in ammonium chloride, and 13 per cent of that in ammonium sulfate were oxidized, while from 0 to 4.8 per cent was nitrified in the dark. He claimed that in 160 hours 12.2 per cent of the ammonium phosphate, and 6.7 per cent of the ammonium chloride were oxidized, while in the dark the amount was 0 to 1.5 per cent.

A comparison of these three salts is made in table 2, and additional details are given in tables 3, 4, and 5. The figures in the latter tables are the total amounts of nitrous and nitric nitrogen in the cultures at the end of the test. The quantities in the soil alone must be deducted and the nitric and nitrous nitrogen added together in order to get the quantities produced from the ammonium salts as given in table 2.

Tables 2 and 4 show that practically no oxidation occurred in any of the cultures containing ammonium chloride exposed to the sunshine. Oxidation of ammonium sulfate in the sunlight occurred in 5 of the 24 cultures (tables 2 and 3). Oxidation of diammonium phosphate occurred in the sunlight in most of the unsterilized soils. The average for 24 tests in the sunshine is 3 per cent of the nitrogen of ammonium sulfate oxidized, 0.1 per cent of that of ammonium chloride, and 11 per cent of that of diammonium phosphate. This apparently confirms Dhar's claim that ammonium phosphate oxidized to a greater extent in the sunshine than did the ammonium chloride or ammonium sulfate. In the dark, the oxidation—45 per cent, 27 per cent, and 50 per cent for sulfate,

chloride, and phosphate respectively—was much greater than in the sunshine. Thus the difference in the oxidation of the ammonium sulfate and of the ammonium phosphate appears to be really due, not to greater oxidation by the sunlight, but to a lower inhibiting effect of the light on bacterial activity, possibly because the ammonium phosphate cultures were exposed to less intensive light than were the cultures containing the other two salts.

EFFECT OF SUNSHINE ON NITRIFYING ORGANISMS

If photonitrification occurs, it should take place in sterilized soils. Tables 2, 3, 4, and 5, show that no nitrification occurred either in sunshine or in darkness with most of the sterilized samples: the amount of oxidation is only a few parts per million where it occurs, with the exception of one culture of soil, No. 36370. This culture was probably not completely sterilized. The amount of nitrates produced in the few other sterilized samples was small and may have been due to contamination, during the period of exposure, with dust which would be mixed into the soil during the daily stirring.

Nitrification occurred in all the cultures which were kept in the dark and contained the nitrifying organisms, either originally in the soil or added by the inoculating liquid. Evidently the nitrifying organisms produced the nitrification. The cultures kept in the sun invariably produced less nitrates than did the corresponding cultures kept in the dark. The sunlight decreased the activity of the nitrifying organisms and reduced the nitrification. Apparently the sunlight either killed the organisms or prevented them from multiplying. According to W. M. Gibbs (5), cultures of both nitrosomonas and nitrobacter are so sensitive to light that when inoculated in cultures in the laboratory neither organism will produce oxidation unless protected from light; direct sunlight causes complete destruction of the organisms, whereas diffused light merely hinders their activity unless the period of exposure is long, which proves fatal.

EFFECT OF SUNSHINE ON NITROUS AND NITRIC ORGANISMS

The discussion so far has concerned only the total oxidation of the ammonia. Both nitrous and nitric nitrogen were estimated, as shown in tables 3, 4, and 5. It is generally accepted that ammonia must pass through the nitrite stage before it is converted to nitrates. If such is the case, both the nitric and nitrous organisms must have been destroyed, or prevented from developing, by sunlight, since the sum of the nitric and nitrous nitrogen is much less in the cultures exposed to light than in those kept in the dark.

Tables 3, 4, and 5 show that a number of cultures exposed to the light contain a little more nitrites than do the corresponding cultures protected from light. In some cases, no nitrites occur in the cultures kept in the dark but they occur in appreciable amounts in the cultures exposed to sunlight. It appears from these results that the nitrous organisms are more resistant to light than are the nitric organisms. The difference is small, however, since the quantities of nitrites produced in the cultures exposed to light are small.

DISCUSSION

It must be pointed out that the temperature (taken daily at 3–4 p.m. except Sunday) was higher in the cultures exposed to sunlight than in the covered ones near by. The average differences (tables 3, 4, and 5) were from 5.2 to 3.2°C. for the cultures containing ammonium sulfate exposed in May, June, and July, but the average differences were only about 1 to 1.5° for those containing ammonium chloride and ammonium phosphate exposed in September and October. The temperature in all cases was higher than is believed to be the optimum (35°C.); nevertheless, nitrification was high in many of the cultures where the organisms were present and which were protected from the sun.

The work here presented does not entirely exclude the possibility of a slight photochemical oxidation of ammonium salts in the soil during long periods of sunlight. It shows, however, that under the conditions described at College Station, Texas, the photochemical oxidation, if it occurs, is vastly inferior in importance to the nitrification caused by organisms. It also shows that direct sunlight is injurious to the microörganisms. The activity of the nitrifying organisms may be decreased or entirely eliminated on soil surfaces exposed to direct sunlight. The surface exposed to light is, however, relatively very small compared to the mass of the soil, so that the injurious action of light upon bacterial nitrification is probably insignificant from a practical point of view.

SUMMARY

To determine how light affects nitrification, samples of soils exposed to sunlight for 4 weeks and stirred daily to present new surfaces to the light were compared with corresponding samples protected from the light. On an average of 72 comparisons conducted on six soils, each with four treatments and three different salts of ammonia, 5 per cent of the ammonia was oxidized in sunlight and 41 per cent in the dark. The oxidation of the added ammonium salt was in all comparisons lower in the cultures exposed to the light than in corresponding cultures that were kept in the dark.

The decrease in nitrification in the sunlight was lower with ammonium phosphate than with ammonium chloride or ammonium sulfate, though this may have been due to the exposure's being made at a period when the light was less intense than when the other two soils were exposed. Nitrites in small amounts were found in cultures exposed to the light, whereas little nitrites or none was found in the cultures kept in the dark, all the nitrogen oxidized being changed to nitrates. This indicates that the nitric organisms are less resistant to light than are the nitrous organisms.

Both nitrous and nitric organisms were rendered inactive or were destroyed by sunshine in cultures of soils exposed to sunlight and stirred daily.

If photonitrification occurs, it should take place in sterilized soils. No evidence of nitrification due to sunlight was found in any of the sterilized soils.

A few of the unsterilized cultures of soils exposed to sunlight and to which ammonium salts were not added contained slightly more nitrates or nitrites

than did the corresponding soils kept in the dark, but sterilized samples of the same soil, as well as sterilized soils inoculated with nitrifying organisms, did not contain more nitrates when kept in the sun than when kept in the dark. The increases in the few cultures mentioned were probably duè to differences in bacterial activity and not to photonitrification.

Although this work does not entirely exclude the possibility of photonitrification, it shows not only that photonitrification must be of little or no practical importance but also that sunlight greatly decreases the nitrification caused by bacterial action, when the bacteria are directly exposed to the sunlight. Since only a small portion of the soil is exposed to direct sunlight, the destructive action of sunlight upon nitrifying organisms is not likely to be of agricultural importance.

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CHARACTERISTICS OF CERTAIN BACTERIA BELONGING TO THE AUTOCHTHONOUS MICROFLORA OF SOIL[1]

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In an article published in 1917 (3) the senior author discussed the great abundance of non-spore-forming bacteria in soil, especially of those which were designated at that time as "slow-growers." Their significance in soil was then stated to be problematic but undoubtedly important. Since then special attention has been given in this laboratory to this group of bacteria, and two or three subsequent papers have been published concerning them. In these later papers the original term "slow-growers" has been dropped because it has been realized that the bacteria do not grow slowly in soil. Instead, they have been called the "punctiform-colony formers."

DISCUSSION OF PREVIOUS WORK

Meanwhile, Winogradsky resumed his work in soil bacteriology and, beginning his investigations with a microscopic study of soil, noticed the large number of organisms present that did not seem to grow on his plates. A few translations from one of his first papers on this subject (9) are quite to the point:

In studying the progress of decomposition of organic matter in the soil, one comes to distinguish two successive periods, as well as two great groups of organisms which correspond to them, or which preside over them.

The first is characterized by relatively rapid activities of which the successive phases gradually grow more slowly as the organic molecule becomes less and less liable to attack by the majority of the ferments. The turn of the second group comes promptly after the organic matter, enriched by unutilized carbon, is transformed into a brown or black substance which is an intimate part of arable soil. The course of the phenomenon is then definitely changed. It assumes the character of slow combustion, much studied by the soil chemists, but of which soil biologists have not yet taken up the study.

One already knows a considerable number of bacterial species belonging to the first group; as they lend themselves well to culture in the conventional media rich in nitrogenous material, they have been isolated without difficulty. The current methods, based exclusively on cultural technique, do not reveal the others which are to be regarded as the types actually representing the soil microflora.

Now, in following the origin of the former and their activity, one concludes that they penetrate periodically into the soil from outside with the organic matter in the course of decomposition (bodies of animals, plant residues, buried manure, etc.), and that they do nothing

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95

in soil except carry on the activities already under way, favored only by the protection which they find there, by the humidity, and by the bases which they can borrow therefrom; but they live only at the expense of their preferred nutrients, recently incorporated with the soil, and they carry out their activities only as long as the latter last, after which they pass into a resting stage, which can continue indefinitely in the soil.

If one, therefore, decides (which seems logical) to consider as *biologically normal* a soil which does not contain any organic substance in the course of decomposition, nor in general any fermentable material, such as proteins or amino compounds, carbohydrates, higher alcohols, organic acids, etc. (i.e., no other substances as organic food except the brown or black material), then one can conclude that the group in question will be completely inactive; it cannot be a part of the microflora of active soil.

By the direct method which we have recommended, which is based on the microscopic study of soil, it is easy to prove this point of view. The most careful microscopic examination of normal soil, indeed, does not reveal in the vegetative condition any microörganisms active upon the substances cited above: neither bacilli (which one believes so characteristic of good soil) nor yeasts, nor fungi, etc. These organisms are present only in resting stages which, sometimes on account of their limited number and sometimes by their minute size and difficult stainability, generally escape observation in the microscopic study of the soil.

If one wishes to make them multiply, it is only necessary to add to the soil an appropriate organic substance of the category which we have mentioned. The effect is immediate but temporary, for this multiplication stops and merely leaves in the soil a new reserve of resting forms as soon as the substances which have brought about their multiplication are exhausted.

These very simple experiments are most convincing. They show clearly that the activity of this group of microörganisms is distinctly associated with the presence of substances foreign to normal soil and consequently their activity can only be intermittent and interrupted as it is (so far as concerns field soil) by resting periods longer than those of activity.

Hence in leaving out of account this group of microörganisms, widespread in most media but not in that of the soil, one comes to believe that the true microflora of normal arable soil is still unknown and that for this reason it is necessary to study the agents of slow combustion so characteristic in this natural medium.

The application of the direct method, indeed, permitted us to discover at the very beginning of our research in the laboratory of l'Institut Pasteur de Brie Comte-Robert a number of species still unknown living in an arable soil not having received any fertilizer for several years. . . .

These rather numerous observations lead us to look at this group as consisting of *"organismes humicoles"* especially adapted, therefore, to the medium in which they live; and it is these to which we refer in grouping them under the designation of the *autochthonous microflora* of arable soil.

In these same papers (9, 10) Winogradsky indicates his intention of doing more extensive work on the *autochthonous microflora* of soil. His interests of recent years, however, have been confined largely to other groups of bacteria, and his later publications, therefore, have not dealt with this particular group. It is, accordingly, concluded that he is still uncertain just what the nature of these bacteria may be. In his early papers he refers to them as organisms that cannot be cultivated on ordinary culture media. He assumes this because of the fact that they are largely round or nearly round organisms which he calls "cocci," while very few of the cultures obtained from soil on ordinary media are coccus forms.

The observation a few years later (5) as to the great abundance in soil of *Bacterium globiforme* Conn tends to make it a little less certain that the or-

ganisms seen under the microscope by the direct technique cannot be obtained by cultural methods. This organism, as shown in its original description, appears as a rod for the first day or two after being transferred to a fresh medium, but subsequently becomes spherical, and practically always appears in soil as a coccus. This organism may, therefore, well comprise a large part of Winogradsky's *autochthonous microflora*. Furthermore, the writer ventures to differ to some extent as to the prevalence of the spherical forms, at least so far as concerns the soils examined in the United States. Among the soils studied here by the direct method, very small rods, so short as to resemble cocci, seem to be more numerous than truly spherical forms. Furthermore, many of the small rods and coccoid bodies seen in such preparations are actually *Actinomycetes* spores. These latter are practically indistinguishable from bacteria in such preparations unless one stains slides that have been in actual contact with undisturbed soil, as did Rossi (7) and Cholodny (2), in which preparations the spores of these organisms can be recognized by their orientation.

For all of these reasons there seems some question about Winogradsky's conclusions that none of the *autochthonous microflora* have been isolated by cultural methods. As a matter of fact, it seems entirely probable that these organisms are for the large part identical with the group previously described in this laboratory as "slow-growers" or the "punctiform-colony formers."

PROBABLE RELATIONSHIPS OF THE ORGANISMS

The present paper is written on the assumption that the *autochthonous microflora* discussed by Winogradsky consists of *Bact. globiforme* and other closely related forms which in soil resemble cocci. It is, accordingly, of interest to consider what the relationships of these organisms to other bacteria may be. As long as it was assumed that they were forms that could not be isolated from soil, no study could be made of their relationships to other bacteria. Assuming, however, that they correspond to the aforementioned group of bacteria that have been isolated, a systematic study of them becomes possible.

The organisms considered in this publication are non-spore-forming organisms, and during recent years several efforts have been made to classify them. The earliest classification, which has since been regarded as unsatisfactory, was on the basis of gelatin liquefaction and chromogenesis. Finally in 1925 (4) it was shown that the bulk of these organisms consisted of two groups, the first simple rods, and the second what were then called "coccus-forming-rods." Not much progress has been made since that date in classifying the bacteria, although in 1928 it was concluded (5) that the second of these two groups consists of only one organism, *Bacterium globiforme*, which appears as a rod in cultures only 1 or 2 days old but as a coccus in older cultures.

The classification of these bacteria is difficult because they have so few features by which they can be characterized. The simple rods differ very little from one another in morphology or apparently in their physiology. The or-

dinary bacterial tests, such as ability to ferment various sugars, do not give satisfactory results when applied to these bacteria, because of the difficulty in obtaining end products in measurable quantities. Thus, all of these bacteria seem to utilize various sugars and to produce a small amount of acid therefrom; but the amount of acid is so small that it can almost never be detected in a highly buffered medium and, even when it is studied in a synthetic medium, its appearance is such an irregular phenomenon that it hardly proves a satisfactory basis for characterization.

This being the case, the writers are still uncertain whether to call all the simple rods that produce punctiform-colonies on gelatin, members of a single species or to consider that there is a great variety of such bacteria in soil. The greatest probability seems to lie with the latter assumption, but just how many species there may be or how they differ from one another is still a puzzling question.

It is likewise uncertain just where to place these organisms in any of the generally accepted classifications of bacteria. Before the peculiar morphology of *Bact. globiforme* was discovered it was assumed that some of these bacteria belonged among the cocci and some among those short rods that have been gathered together in Bergey's Manual (1) under *Achromobacter*. As a matter of fact, the latest edition of this manual places *Bact. globiforme* in the aforementioned genus. This name for it is not accepted by the writers, however, as it is felt that further study of this and similar organisms must be made before deciding whether to put them closest to the rods or the cocci. The fact that *Bact. globiforme* grows in soil in a spherical form suggests close relationships to the cocci. On the other hand, the similarity of this organism in its physiology to the other punctiform-colony forming bacteria of soil (and in many respects, indeed, to the legume nodule organisms) suggests relationships to the *Bacteriaceae*.

On account of this puzzle, the writers prefer to retain the name originally given to this organism. This is done in recognition of the fact that no such genus as *Bacterium* is recognized in Bergey's Manual and that, accordingly, an organism whose relationships are doubtful might well remain in the old genus *Bacterium* until it has been more carefully studied.

It is largely to give it this needed study that *Bact. globiforme* has received such a large share of the writers' attention in recent years; but there are other reasons also for the intensive study that has been given the organism. When first observed it appeared interesting because of its apparent ability to change back and forth from a rod to a coccus form. Still greater interest was added to it when it was discovered that none of the cultures on hand in the writers' laboratory had been isolated from sod soils, although among the simple nonspore-forming rods in the same collection of cultures as many had been isolated from sod as from cultivated soil. Even more significant was the observation that this organism appeared to be more abundant in the better soils than in those of low productivity. This last observation suggested that *Bact.*

globiforme either had some causal relation to the productivity of soil, or else those conditions favoring the growth of plants likewise stimulate the development of this organism.

It has since been found that *Bact. globiforme* in its physiology is quite typical of the whole group of punctiform-colony formers in soil. This fact, together with its peculiar morphology which makes it easy to recognize, seems to indicate this organism as a good one to be selected as a typical representative of the group under investigation.

FUNCTION OF THE ORGANISMS

In calling the bacteria which predominate in normal soil "autochthonous (i.e., *indigenous* or *native*) microflora," Winogradsky has certainly selected a much more appropriate name than either of those previously employed by the writer. In referring to them, however, with the adjective "*humicoles*" or "*humivores*" (10), it is not so certain that his terminology is correct. These two latter terms indicate, respectively, "living on humus" or "feeding on humus." In proposing these names, therefore, he is indicating the functions of the organisms in question in spite of the fact that he has not isolated them or learned their behavior. There is, on the other hand, an entirely different function that such organisms as these might perform and yet appear in exactly the conditions under which Winogradsky finds them.

This other possible function is as follows: In the course of the active decomposition of organic matter such as is carried on by the other group of organisms (called by Winogradsky *zymogenous*), much soluble nitrogen is formed as well as the insoluble brown or black material on which Winogradsky assumes the autochthonous organisms feed. This soluble form of nitrogen consists largely of nitrates, ammonium salts, and amino compounds. It is entirely possible, therefore, that a group of organisms may exist in the soil which seize upon these soluble forms of nitrogen and build them up into body protein. Inasmuch as all arable soil is quite sure to be supplied with a constantly renewed source of soluble compounds of this sort, it would be a fairly constant source of food for such organisms as these, and hence they would be uniformly present with much less fluctuation in numbers than the organisms of the zymogenous type.

Organisms carrying on the activities just mentioned would, of course, require in addition to this soluble nitrogen some source of carbon as well as a source of energy. A source of both carbon and energy might well be found in the humic constituents upon which Winogradsky assumes them to live. On the other hand, they might utilize for this purpose, either directly or indirectly, the celluloses, hemicelluloses, or starches occurring in soil. If they utilize such carbohydrates indirectly, it must be assumed that they live in association with the cellulose-decomposing bacteria and obtain their carbon and energy from the products of the hydrolysis brought about by these other forms.

With this thought in mind it becomes of interest to review the facts so far

known as to the activities of *Bact. globiforme* and the other punctiform-colony bacteria in soil to see how well they could play the aforementioned rôle. Although the function of these organisms is still a matter of speculation, certain inferences can be drawn from their distribution and from their nutritive requirements.

Especially significant in this connection are the results previously brought out (5) indicating those nitrogen sources available to *Bact. globiforme*. It was shown in the previous publication to be capable of obtaining its nitrogen from nitrate, ammonium salts, amino compounds, or even higher forms of nitrogen such as those present in bacteriologic peptones. Apparently it is capable of utilizing almost any source of soluble nitrogen, either organic or inorganic. This fact has been established both by culture in ordinary laboratory media and by observing which nitrogen compounds enable it to grow vigorously in soil which does not naturally contain any nitrogen available for the organism. In other words, so far as concerns its nitrogen relationships, this organism and presumably the others under investigation might well play the rôle discussed in the foregoing.

As to sources of carbon and energy available to the organism, the work mentioned has shown that stimulation of its growth can occur in soil upon the addition of certain organic acids without other added carbon compounds. Probably, therefore, such compounds can furnish bacteria of this group with their carbon and energy. Most readily available, however, of all carbon sources prove to be the sugars, especially glucose. At first thought it seems strange that those bacteria apparently the most numerous in soil should have a predilection for the simple sugars which are never found in soil. On further consideration, however, the situation is not so unlikely as it might seem. The decomposition of cellulose, pentosans, and starch is occurring constantly in the soil, and at an early stage of this decomposition there is almost sure to be production of some hexose or pentose. That these lower carbohydrates do not accumulate in soil may well be because such organisms as those here discussed are always present to consume the sugars as rapidly as they are formed. Thus, organisms capable of utilizing minute quantities of sugar may well find their place in Nature's scheme for the carbon and nitrogen transformations in soil.

Assuming this to be the function of the organisms under investigation, an intensive study seemed called for to learn just how economically they are able to utilize the simple sources of carbon and of nitrogen available to them. This was the object of the work discussed in the following. In this work glucose or sucrose was chosen as a source of carbon, and ammonium phosphate as a source of nitrogen. Analyses were made to determine to what extent the nitrogen and the carbon in these compounds were utilized for building cell tissue and for the production of energy.

Three cultures were used in this work: a strain of *Bact. globiforme* which had been kept as a stock culture in the laboratory since the species was first de-

scribed; also two undetermined simple short rods here denoted only by the laboratory numbers L 2i₄ and III 2i₈. These last two cultures were included largely for purpose of comparison with *Bact. globiforme.*

Utilization of sugars

In table 2 of the 1928 publication on this subject (5) are given certain preliminary figures as to the amount of sugar consumed in a liquid medium containing 1 per cent glucose. The results then obtained were noticeably discrepant. It was concluded that the discrepancies were caused by the difficulty of determining sugar accurately when the difference between the initial quantity and the amount left after the growth of the culture is small. In order to obtain a higher percentage of sugar consumed, an experiment was carried on to determine the minimum quantity of glucose that could be added to a culture medium and still permit approximately the maximum of growth and production of carbon dioxide. This work was done in Eldredge tubes containing

TABLE 1

CO_2 production in liquid medium with varying percentages of glucose

Incubation—14 days

CULTURE	TEST NUMBER	CO_2 PRODUCED FROM MEDIA CONTAINING GLUCOSE IN PERCENTAGES AS FOLLOWS*						
		0.02 per cent	0.05 per cent	0.1 per cent	0.2 per cent	0.5 per cent	1 per cent	1.5 per cent
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Bact. globiforme........	1		10.5	17.5	23.5	25.2	**27.9**	
Bact. globiforme........	2	3.6	10.4†	15.9	**22.1**	19.5†	18.9	17.2
Unidentified ⎰ L 2i₄....	3	5.0	9.7	13.3	10.5	13.0	**15.3**	14.0
short rods ⎱ III 2i₈...	4	3.5	11.7	**19.7**	14.1	16.3	11.5†	12.5

* All determinations in triplicate except those marked †, which were in duplicate only.

20 ml. of medium in one arm and the same quantity of 0.1 N barium hydroxide in the other. The basal medium employed was as follows:

$(NH_4)_2HPO_4$...	1.0 gm.
KCl..	0.2 gm.
$MgSO_4 \cdot 7H_2O$..	0.2 gm.
H_2O..	1,000.0 ml.

To this basal medium were added different quantities of glucose from 0.02 per cent up to 1.5 per cent. The results of the four tests which were run for this purpose are given in table 1. In the line recording the results of each test the maximum CO_2-production is indicated in bold face type. It will be seen that the percentage of sugar giving the maximum amount of CO_2 varied considerably in the different tests, as it was occasionally as high as 1 per cent and in one instance as low as 0.1 per cent. It is noticeable, however, that very little tendency to increase was observed with concentrations of glucose greater

than 0.1 per cent. As a result of these figures it was concluded that 0.1 per cent or 0.2 per cent would be about the best quantity to employ in order to obtain the highest percentage of sugar utilization. As a matter of fact, the quantity employed in the following experiments was 0.15 per cent.

In connection with these results it is interesting to compare the respiration figures recently published by Georgi and Wilson (6) concerning the legume nodule organism. In this work they employed an agar medium in Eldredge tubes and determined not only the amount of carbon dioxide given off but the amount of oxygen consumed. Of special interest is the fact that, with quantities of glucose varying from about 0.6 per cent up to about 1.6 per cent, they found the amount of carbon dioxide produced to vary from about 10 to 20 ml. (e.g., from 20 to 40 mgm.) for each tube. Such results are similar to those obtained in the present work with a different kind of soil bacteria. Georgi and Wilson found that between 60 and 80 per cent of the carbon in the glucose consumed was converted into carbon dioxide.

The experiments which follow indicate how nearly these last-mentioned figures of Georgi and Wilson agree with results obtained with *Bact. globiforme* and the related organisms in soil.

In seven different experiments Eldredge tubes were prepared with 25 ml. of the medium in the culture arm and in the other arm 15 ml. of 0.1 N barium hydroxide diluted with distilled water up to 20 ml. The cultures were incubated for 2 weeks, and the barium hydroxide was then titrated to determine the amount of CO_2 given off. The contents of the culture arm were then placed in a centrifuge for about 2 hours, after which the supernatant fluid was decanted from the centrifuge tube and a sugar determination made of it by the macro method of Shaffer and Hartmann (8). Meanwhile, the growth which remained in the centrifuge tube was washed in the centrifuge two or three times, then removed, dried, and weighed. It was desired by this procedure to obtain the following information: amount of sugar utilized; percentage of the carbon in that sugar converted into CO_2; amount of carbon utilized in building up the bacterial cells, on the assumption that the growth obtained on centrifuging was mostly protein and contained approximately 50 per cent carbon; and finally, the percentage of carbon in the sugar not accounted for in either of these ways. In connection with the aforementioned assumption of 50 per cent carbon in the dried bacterial growth, it should be remarked that this was obtained from the estimate that if this substance were all protein it would contain about 53–54 per cent carbon. Presumably, however, a certain amount of carbohydrate and a certain amount of ash would be present, so that no accurate estimate as to the percentage of nitrogen in the cell substance can be made; but as the quantities of material other than protein were probably not large, the 50 per cent assumption seemed a safe one to make. It was not expected, however, that by any means all the carbon could be accounted for in these experiments; even if the organisms were capable of utilizing the glucose without the production of by-products, it would be very unlikely that

TABLE 2

Sugar consumption by Bact. globiforme

TUBE NUMBER	SUGAR CONSUMED			CO₂ PRODUCED		RATIO: $\dfrac{C \text{ IN } CO_2}{C \text{ IN SUGAR}}$
	Quantity	Per cent	C-content	Quantity	C-content	
Experiment 1. Initial sugar: 32.6 mgm. glucose per tube						
	mgm.	*per cent*	*mgm.*	*mgm.*	*mgm.*	*per cent*
1	19.7	60.4	7.9	26.0	7.1	90
2	28.2	86.5	11.3	16.1	4.4	39
3	20.6	62.2	8.25	19.8	5.4	65.5
4	23.1	70.8	9.25	20.7	5.65	61
5	24.2	74.3	9.7	18.5	5.05	52
6	27.4	84.0	10.9	22.2	6.05	55.5
7	27.0	82.8	10.8	21.3	5.8	53.5
8	25.1	77.0	10.1	19.6	5.35	53
Total for 200 ml.........	195.3	74.8	78.2	164.1	44.8	57.3

Bacterial growth in 200 ml.: 40 mgm.; estimated C-content: 20 mgm.
Total C accounted for: 64.8 mgm. = 83 per cent of C consumed

Experiment 2. Initial sugar: 37.5 mgm. glucose per tube

1	7.5*	20	3.0	19.4	5.3	
2	33.1	87.2	13.2	22.9	6.25	
3	30.9	82.2	12.4	19.1	5.2	
4	3.0	8	1.2	19.4	5.3	
5	17.0	45.3	6.8	18.7	5.1	
6	36.7	97.7	14.6	23.8	6.5	
7	12.7	33.8	5.1	18.7	5.1	
8	36.7	97.7	14.6	18.9	5.25	
Total for 200 ml.........	177.6	59.2	70.9	160.9	44.0	62.1

Bacterial growth in 200 ml.: 48 mgm.; estimated C-content: 24 mgm.
Total C accounted for: 68 mgm. = 97.5 per cent of C consumed

Experiment 3. Initial sugar: 39.6 sucrose per tube

1	24.0	60.7	9.6	15.8	4.3	44.8
2	18.1	45.8	7.25	14.4	3.9	53.8
3	25.2	68.8	10.1	19.1	5.2	51.5
4	16.1	40.8	6.45	13.9	3.8	59.0
5	14.1	35.7	5.65	13.9	3.8	67.3
6	16.6	42.0	6.65	17.8	4.85	73.0
7	17.0	43.0	6.8	15.8	4.2	61.8
8	20.9	52.9	8.4	13.9	3.8	45.3
Total for 200 ml.........	152.0	48.0	60.9	124.6	33.9	55.7

Bacterial growth in 200 ml.: 52.7 mgm.; estimated C-content: 26.4 mgm.
Total C accounted for: 60.3 mgm. = 99 per cent of C consumed

TABLE 2—*Concluded*

TUBE NUMBER	SUGAR CONSUMED			CO₂ PRODUCED		RATIO: $\dfrac{\text{C IN CO}_2}{\text{C IN SUGAR}}$
	Quantity	Per cent	C-content	Quantity	C-content	
Experiment 4. Initial sugar: 33.2 glucose per tube						
	mgm.	*per cent*	*mgm.*	*mgm.*	*mgm.*	*per cent*
1	11.6	35.0	4.65	13.7	3.7	79.5
2	13.6	41.0	5.45	13.4	3.65	67.0
3	7.4	21.3	2.9	10.3	2.8	96.5
4	7.7	24.2	3.1	10.8	2.95	95.0
5	9.2	27.7	3.7	11.9	3.25	87.8
6	12.0	36.2	4.8	14.1	3.85	80.5
7	8.5	25.6	3.4	11.2	3.05	89.5
8	12.0	36.2	4.8	14.7	4.0	83.5
Total for 200 ml..........	82.0	30.8	32.8	100.1	27.25	83.1

Bacterial growth in 200 ml.: 34.0 mgm.; estimated C-content: 17 mgm.
Total C accounted for: 44.2 mgm. = 135 per cent of C consumed

* The great variation in the individual determinations in this experiment are thought to be analytical errors. See text.

all the growth could be separated from the medium and, therefore, all the carbon thus utilized determined.

The results of these experiments are given in tables 2 and 3—table 2, those with *Bact. globiforme*, and table 3, those with the unindentified short rods. Before any conclusions are drawn, it seems well to mention instances where the work may possibly be in error. In experiment 2 of table 2, for example, the sugar analysis was made under conditions which caused extreme variation, as shown by the figures given. It was planned at first to discard the results of this experiment entirely; but the average figures proved to agree so well with those of other experiments that they are given for whatever they may be worth. In the second place, experiment 4 of table 2 showed an unusually low figure for sugar consumption in every instance, so low, in fact, that apparently 135 per cent of this figure was accounted for in the products of growth; although no other evidence of analytical mistakes was obtained in this experiment, it is plain from the results that some such error must have occurred. Finally, it must be explained that experiments 1 and 3 of table 3 were carried on with a slime-producing organism, and it is known that some of this slime was lost in the course of washing the cells, and accordingly no estimate could be made as to its weight and carbon content.

Even when these possibilities of error are taken into account, the figures do bring out certain points of significance. It will be seen, for example, that the amount of sugar utilized, leaving out experiment 4 with *Bact. globiforme*, varied only from 48 to 74.8 per cent of that initially present. This agrees very closely with the 60 to 80 per cent found by Georgi and Wilson in the

TABLE 3

Sugar consumption by unidentified short rods

TUBE NUMBER	SUGAR CONSUMED			CO₂ PRODUCED		RATIO: C IN CO₂ / C IN SUGAR
	Quantity	Per cent	C-content	Quantity	C-content	

Experiment 1. Culture no. III 2i₈.* Initial sugar 34:0 mgm. glucose per tube

	mgm.	*per cent*	*mgm.*	*mgm.*	*mgm.*	*per cent*
1	19.8	58.2	7.9	12.6	3.44	43.5
2	18.1	53.2	7.25	12.6	3.44	47.4
3	15.2	44.7	6.1	10.6	2.89	47.3
4	13.5	39.7	5.4	10.8	2.95	54.6
5	15.5	45.5	6.2	10.8	2.95	42.6
6	16.7	49.1	6.7	10.1	2.76	41.2
7	13.8	40.6	5.5	10.3	2.81	56.1
†Total for 200 ml........	128.7	47.3	51.5	88.9	24.3	47.2

Bacterial growth in 200 ml.: 40 mgm.; estimated C-content: 20 mgm.
Total C accounted for: 44.3 mgm. = 86 per cent of C consumed

Experiment 2. Culture no. L 2i₄. Initial sugar 33.4 mgm. glucose per tube

1	18.3	54.8	7.3	16.05	4.4	60.3
2	16.7	50.0	6.7	14.75	4.05	60.5
3	17.6	52.6	7.05	13.85	3.8	53.9
4	20.6	61.7	8.25	14.5	3.95	47.8
5	16.7	50.0	6.7	14.95	4.1	61.3
6	23.0	68.9	9.2	17.6	4.8	52.2
7	16.4	49.0	6.55	14.5	3.95	60.3
8	16.4	49.0	6.55	13.4	3.65	55.7
Total for 200 ml.........	145.7	54.5	58.3	119.6	32.7	56.1

Bacterial growth in 200 ml.: 60.4 mgm.; estimated C-content: 30.2 mgm.
Total C accounted for: 62.9 mgm. = 107.8 per cent of C consumed

Experiment 3. Culture no. III 2i₈.* Initial sugar 34.1 mgm. glucose per tube

1	15.2	44.5	6.1	16.5	4.5	73.8
2	19.8	53.1	7.95	15.6	4.25	53.5
3	16.5	48.4	6.6	13.4	3.65	55.3
4	17.1	50.2	6.85	15.2	4.15	60.6
5	15.1	44.3	6.05	14.3	3.9	64.5
6	15.2	44.5	6.1	14.5	3.95	64.8
7	17.1	50.2	6.85	13.0	3.55	51.8
8	14.8	43.4	5.9	14.3	3.9	66.2
Total for 200 ml.........	130.8	47.9	52.40	116.8	31.85	60.8

Bacterial growth in 200 ml.: 24.3 mgm.; estimated C-content: 12.1 mgm.
Total C accounted for: 44 mgm. = 84 per cent of C consumed

* A slime-producing organism.   No estimate made of the amount of carbon in the slime.
† One tube of this series was lost but the figures in this line are computed on the basis of
; tubes (i.e., 200 cc.).

case of the legume nodule organism. Also, the ratio of carbon in the CO_2 produced, to the carbon in the sugar utilized (also leaving out experiment 4, table 2) was never below 47.2 per cent or above 62.1 per cent. Georgi and Wilson found this ratio to be from about 40 per cent to about 90 per cent in the case of the organisms they were studying; and this gives further evidence as to similarities between the organisms here under investigation and those of the legume nodules. Finally, it will be seen from these tables that the total carbon accounted for amounted in every experiment to over 80 per cent of the sugar consumed.

The conclusion drawn from these data is that organisms of this punctiform-colony group probably utilize sugars for two purposes only. Part of the sugar is used as a source of carbon and is built up into their cell tissue. Part of it is used as economically as possible as a source of energy and for this purpose is oxidized completely into carbon dioxide. Any sugar that is present in excess of the needs of the organisms for these two purposes remains unchanged in the medium. Thus these bacteria are characterized by an extremely economical utilization of sugar.

One interesting corollary of this fact is that since no by-products are produced from the sugar, the only changes in reaction that can occur are from the CO_2 produced and from the action of bacteria on ingredients other than the carbohydrate. This makes it difficult to devise a simple test, such as acid production, by means of which the action of the bacteria on sugars can be observed. The acid formed from the CO_2 is likely to be insufficient to make itself evident unless the medium is quite free from buffer compounds. In the latter case, changes of reaction due to other causes (such as greater utilization of anions than cations, or *vice versa*) may be so great as to obscure production of acid from the sugar.

Utilization of ammonium salts

As has been stated, ammonium salts are among the preferred sources of nitrogen for *Bact. globiforme*. It became of interest, accordingly, to make an analytical study of the utilization of ammonium salts by this organism. Two experiments were conducted for this purpose. Ammonium phosphate was chosen and the organism was grown on the following medium:

$(NH_4)_2HPO_4$	2.0 gm.
KCl	0.4 gm.
$MgSO_4 \cdot 7H_2O$	0.4 gm.
Glucose	20.0 gm.
Agar (air-dried)	30.0 gm.
H_2O	2,000.0 gm.

The medium was distributed among several containers in which it was allowed to harden in such a position that there would be as large a surface as possible to inoculate. About two-thirds of the containers were inoculated and the others were kept as checks. After 2 weeks of incubation the growth

was carefully removed from the surface of the agar and the nitrogen was determined by the modified Kjeldahl method of the Association of Official Agricultural Chemists. At the same time ammonia determinations were made (by distillation by magnesium oxide) on 200-gm. samples of the inoculated medium after the growth had been removed, and of the uninoculated medium which had been incubated under exactly the same conditions as the inoculated containers.

The object of these determinations was to learn what proportion of the ammonium phosphate could be utilized by this organism and what percentage of that which it did use was converted into cell tissue.

Experiment 1. In the first experiment the containers used for the medium were large test tubes about 30 mm. in diameter. In each tube 30 ml. of the medium was allowed to harden while in a slanted position. The total weight of the inoculated medium after incubation was 1,326 gm. Aliquots of this and of the uninoculated medium were analyzed, and all computations were figured to the basis of the entire 1,326 gm. The results of the analysis were as follows:

Ammonia N in uninoculated medium........................... 239.3 mgm.
Ammonia N in inoculated medium............................ 90.5 mgm.
Ammonia N consumed....................................... 148.8 mgm.

Dry weight of bacterial growth.............................. 1.6738 mgm.
Total N in bacterial growth................................. 118.2 mgm.

Percentage of ammoniacal N consumed appearing as total N in
  growth... 79.1 per cent

Experiment 2. The second experiment differed from the first in two respects. The containers employed were 500-ml. wide-mouth Erlenmeyer flasks, and 100 ml. of the agar was placed in each flask. The larger surface presented by the agar in these flasks made it much easier to remove the growth after incubation. The other point of difference was that it seemed worth while to make a total nitrogen analysis of the medium from which the growth had been removed. Accordingly, all of the inoculated medium (after the bacterial growth was removed) that was not used for the ammonia analysis was kept in tightly closed flasks in a refrigerator until the next analytical work could be carried on (about 3 weeks). The same method of nitrogen analysis was employed as in the case of bacterial growth, but the large bulk of agar made the digestion process a difficult one. It was first necessary to reduce the bulk of the medium somewhat by partly drying for several days (after adding a small amount of $5N$ H_2SO_4) at a temperature of 55°C. Six or seven weeks were required for the digestion process, as it was not possible to arrange to have digestion kept up continuously night and day. The analytical data obtained were as follows:

Ammonia N in uninoculated medium.......................... 183.5 mgm.
Ammonia N in inoculated medium........................... 3.0 mgm.
Ammonia N consumed...................................... 180.5 mgm.

Dry weight of bacterial growth............................. 2.4472 mgm.
Total N in bacterial growth................................ 127.0 mgm.

Percentage of ammoniacal N consumed appearing as total N in
 growth.. 70.3 per cent

Total N in inoculated medium after removal of growth......... 108.8 mgm.

Total N in growth... 127.0 mgm.
Total N in medium after growth............................ 108.8 mgm.
Total N present after growth............................... 235.8 mgm.

In this experiment the nitrogen balance was not completely figured out because no total nitrogen determination was made on the uninoculated medium. It was known, however, that it contained at the end of the experiment (as has been shown above) 183.5 mgm. ammonia nitrogen and that in addition to the ammonium salt no nitrogen was added to the medium except in the agar and in the way of impurities. On the basis of published analyses of agar it was concluded that at least 35 to 40 mgm. of nitrogen would be added to the medium from this source, so that a total of over 220 mgm. was undoubtedly present before the growth of the bacteria. This checks very closely with the 235.8 mgm. actually found in the inoculated medium after incubation. This at least indicates that none of the nitrogen was lost into the air.

In general it was concluded from these nitrogen analyses that this organism can utilize all the nitrogen present when this element is furnished in the form of 0.1 per cent ammonium phosphate. Nearly 80 per cent of the nitrogen is converted into cell tissue. The remaining portion of the nitrogen consumed, in the instance under investigation, remained in the medium, apparently in some soluble organic form. It is entirely possible that even the nitrogen not accounted for by the cell substance present at the end of the experiment may have been originally converted into cell protein and subsequently decomposed after death of some of the cells.

SUMMARY

A physiological study has been made of some of the organisms previously designated "punctiform-colony formers" because of the small size of their colonies on laboratory media. Reasons are given for considering them as belonging to the group which was seen but not isolated by Winogradsky and called by him the *autochthonous* (i.e., indigenous) *microflora* of soil.

Special attention has been given to one of these organisms, *Bacterium globiforme* Conn, because it is very easy to recognize and because it has been found more numerously in good soils than in those less productive.

In a synthetic liquid medium it has been found that under the conditions

investigated the highest percentage of glucose is utilized if the medium is furnished with about 0.2 per cent of this sugar.

When this medium with 0.15 per cent glucose or sucrose was used and the culture grown in 150-ml. Eldredge tubes containing 25 ml. of the medium, the organisms studied were found to utilize ordinarily between 48 and 75 per cent of the sugar furnished. About 50 to 60 per cent of the carbon in the sugar consumed was converted into CO_2. From the weight of the bacterial growth produced, an estimate was made of the amount of carbon that had been converted into cell substance. When this estimate was added to the amount of CO_2 produced, over 85 per cent of the carbon in the sugar consumed was accounted for. It is, accordingly, concluded that the organisms convert all the carbon of glucose or sucrose into CO_2 and cell substance.

As a similar conclusion has recently been drawn by Georgi and Wilson concerning the legume nodule organism, there seems to be some possibility that this extremely economical utilization of sugar is quite characteristic of a number of soil bacteria.

A study was also made of the nitrogen consumption of *Bact. globiforme* when growing on a solid medium containing 0.1 per cent ammonium phosphate as its sole source of nitrogen. It was found that 60 to 90 per cent of the nitrogen thus furnished was consumed, and that 70 to 80 per cent of that consumed was converted into cell substance. The balance of the nitrogen remained after growth in the medium, no loss of nitrogen being evident.

It is concluded that these bacteria undoubtedly help retain in soil, nitrogen that has been converted by other organisms into a soluble form and, but for the action of this autochthonous microflora, would probably be removed by drainage or utilized by plants. It is still uncertain whether to regard these organisms as ordinarily harmful or beneficial. Perhaps they may be either, according to the conditions under which they are functioning. At times they may be rivals for nitrogen because of their ability to utilize nitrate. On the other hand, under conditions that bring about rapid nitrification in the soil these bacteria may be beneficial. When ammonification is so rapid that ammonium salts tend to accumulate faster than they can be nitrified or nitrates are formed more rapidly than they can be utilized by the plants, there would be considerable loss of nitrogen by leaching but for the action of bacteria in converting it into an insoluble form.

Of these two functions it seems more probable that the beneficial action predominates, to judge from the fact that this group of organisms is ordinarily most abundant in the better soils.

Whether ordinarily harmful or beneficial, it is plain that the organisms studied are well adapted to live under surface soil conditions, as they are very strictly aerobic, can make use of nitrates, ammonium salts, or amino compounds as sources of nitrogen, and can obtain their carbon and energy from such small amounts of sugar that they can undoubtedly utilize for this purpose the minute quantities made available in soil during the digestion of starch and

cellulose. Utilizing simple sources of nitrogen and very small quantities of carbohydrate, they convert inorganic material into cell substance which, undoubtedly, is readily converted into humus upon the death of the bacteria.

The functions of these organisms in soil are evidently of some importance. Whether these activities can be controlled remains for future investigation to show.

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INORGANIC PHOSPHATE IN GREEN PLANT TISSUE AS A MEASURE OF PHOSPHATE AVAILABILITY[1]

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In recent years a number of investigators (4, 5, 7, 9, 12, 13) have suggested that a determination of the content of inorganic or total phosphorus in the sap or tissues of growing plants might throw some light on the phosphate supply of the soil. Thornton (13) in particular has advanced the idea that plants growing in a medium richly supplied with phosphate may be unable to convert the inorganic phosphate into organic form as rapidly as it is absorbed; hence, inorganic phosphate will accumulate within the plant. Conversely, under conditions of deficient supply no such accumulation will occur.

Gilbert and Hardin (5), Emmert (4), Thornton (13), and Pohlman and Pierre (12) have found that, under certain conditions, phosphate fertilization, or an increased phosphate supply in the nutrient medium, causes an increase in the inorganic phosphate of the culture plants. Conversely, it has been shown that plants, when limited in growth by phosphate deficiency, contain reduced amounts of inorganic phosphate. However, none of the previous studies are sufficiently complete to show whether low inorganic phosphate in the tissues of the growing plant is always indicative of a low phosphate supply in the culture medium, and *vice versa*, whether high phosphate in the plant is definitely indicative of an adequate supply. Neither has it been determined definitely just what part of the plant and what stage of growth best reflect the phosphate condition of the nutrient medium, although a number of the aforementioned investigators have devoted some study to this question. In order to obtain further information on these points, pot culture experiments were made with oats on a series of soils varying with respect to phosphate requirement. In addition, a few preliminary tests have been made with citrus. It is the purpose of this paper to report the results of these studies.

EXPERIMENTAL

Method of making inorganic phosphate tests

The method finally adopted is a slight modification of that employed by Thornton (13). Immediately after withdrawal from the soil, the oat plants

were stripped of dead leaf material, the roots were cut off at the crown, and the adhering soil was washed off with distilled water. The excess water was then absorbed between sheets of filter paper. Samples of 0.5 gm. of the part of the plant to be tested were immediately cut into small pieces, transferred to a small glass cylinder, and thoroughly macerated with a blunt glass rod. The macerated material was then transferred with acid-molybdate reagent (0.1 per cent ammonium molybdate dissolved in 0.4 N H_2SO_4) to a 250-cc. Erlenmeyer flask, and enough more of this reagent added to give a final volume of 100 cc. The contents of the flask were shaken vigorously, 10 drops (0.5 cc.) of a 2.5 per cent $SnCl_2 \cdot 2H_2O$ solution added, and after standing 2 minutes the color intensity was compared against Hellige glass color standards (1). In cases where the color was too intense, the solutions were diluted with the acid-molybdate reagent and more $SnCl_2$ solution was added. Chlorophyl introduces an error in the phosphate-molybdate color measurements, especially if the inorganic phosphate content is low; hence the results obtained with material containing relatively much chlorophyl and little phosphate are but roughly quantitative. The small·quantities of inorganic constituents found in most plants are probably insufficient to interfere, provided the method is used as here outlined (2). No important interference by soluble organic constituents was found to occur with the particular plant materials studied.

It is not certain that this test distinguishes quantitatively between inorganic phosphate and certain forms of organic phosphorus. It is conceivable that in an acid medium PO_4 might be formed from certain organic forms by hydrolysis. However, Plimmer's (11) studies on the hydrolysis of nucleic acid, phytin, hexose phosphate, and simple alcoholic phosphates suggest that no appreciable hydrolysis of such of these compounds as may occur in the plant material tested is likely to take place under the conditions of this test. In any case, if an important relationship is found between the phosphate of the nutrient medium and the content of PO_4 in the plant or any of its parts, it is immaterial from a practical point of view whether the distinction between inorganic and organic phosphorus is absolute.

Emmert (3) has outlined a somewhat more exact but less rapid method, and Pierre and Pohlmann (10) have determined inorganic phosphate in the exuded sap of plants.

Pot culture studies with oats

A series of 17 soils varying with respect to phosphate need were assembled, and portions of each were screened, mixed, and potted in 2-gallon earthenware jars.

Duplicate pots of each soil were treated with fertilizers as shown in table 1. The fertilizers were mixed uniformly with the soil in a given pot, the soluble forms being applied in solution and dicalcium phosphate being added to the soil in dry form. In addition $Ca(NO_3)_2$, at the rate of 50 pounds per acre, was applied in solution to the N-treated pots at intervals of 2 weeks during

the growing period. Oats were planted, and when the seedlings had attained a height of 1 inch the stand was thinned to 20 plants per pot.

Different parts of the plants were tested for inorganic phosphate at three stages of growth; namely, the vegetative, heading, and ripening stages. The plants at the first (vegetative) stage were 24 to 26 days old and had attained a height of 6 to 8 inches. At the heading stage plants were chosen in which the panicles were emerging or had just emerged. At the ripening stage the plants were still somewhat green, and the grain was in the early dough stage. Ten plants per pot were used for making these tests, leaving ten plants to be harvested. When approximately mature, these were cut near the crown, dried, and weighed.

Effect of varying phosphate supply.—The oat yields resulting from the differential treatments are recorded in table 2. If it is assumed that sufficient fer-

TABLE 1

Different fertilizer treatments given each soil under pot culture

TREATMENT		GRAMS PER POT	POUNDS PER ACRE		
			N	P_2O_5	K_2O
None	....	....	....	....	....
N	$Ca(NO_3)_2 \cdot 4H_2O$	1.44	50	....	....
NP	$Ca(NO_3)_2 \cdot 4H_2O$	1.44			
	$CaH_4(PO_4)_2 \cdot H_2O$	1.19	50	400	....
	$CaHPO_4 \cdot 2H_2O$	1.62			
NPK	$Ca(NO_3)_2 \cdot 4H_2O$	1.44			
	$CaH_4(PO_4)_2 \cdot H_2O$	1.19	50	400	200
	$CaHPO_4 \cdot 2H_2O$	1.62			
	K_2SO_4	1.25			

tilizer was added to meet crop needs, the yield increase resulting from the NP treatment as compared with the N treatment measures the relative deficiency of P in the pots treated with N alone. In like manner, the relative deficiency of N and K is measured by the yield increases resulting from N as compared with no treatment and NPK as compared with NP. It will be noted that the yield increases produced by phosphate ranged from none to 115 per cent; by nitrogen, from 20.4 to 620.0 per cent; and by K, from no increase to 24 per cent.

Preliminary tests of various parts of the young plants at the first sampling stage showed higher inorganic phosphate in the primary stem and sheath material immediately above the crown, and in the young immature leaves, than in the more mature leaves. However, at this stage both the stem and leafy parts of the plant seemed to be consistent in reflecting the phosphate condition of the nutrient medium. Because of the interfering action of chlorophyl, the tests on leaves are less accurate than those on the stem-sheath ma-

terial immediately above the crown, hence only the results on the latter are recorded in table 3.

As has been stated, the yields resulting from the NP, as compared with the N treatments, afford a measure of the relative deficiency of phosphorus in the N-treated pots. Since the increases from phosphate additions ranged from nothing up to 115 per cent, a measure of the inorganic phosphate level in· the oat plants growing in the N-treated pots to which no phosphate was added should indicate whether at this stage the plant will accurately reflect the phosphate supplying power of these soils. The data bearing on this point are recorded in table 3. It will be noted that there is a striking relationship between the

TABLE 2

Effect of fertilizer treatments on oat yields

SOIL NUMBER	SOIL TYPE	YIELDS RESULTING FROM FERTILIZER TREATMENT*				INCREASE IN YIELD FROM		
		None	N	NP	NPK	N	P	K
		grams	grams	grams	grams	per cent	per cent	per cent
18322	Meloland fine sandy loam	22.5	27.1	26.6	33.0	20.4	None	24.1
18313	Hanford sandy loam	4.4	31.4	30.0	26.4	620.0	None	None
18323	Sierra loam	5.6	23.4	23.5	22.1	318.0	0.4	None
18321	Yolo clay loam	11.7	33.6	34.1	31.9	187.0	1.5	None
18324	Ramona loam	5.8	24.8	25.2	26.1	318.0	1.6	3.5
18325	Placentia loam	5.2	21.6	22.0	18.1	316.0	1.8	None
18319	Yolo gravelly loam	4.5	19.3	19.9	21.7	329.0	3.1	9.0
18314	Hanford loam	4.9	24.0	26.7	28.5	390.0	11.2	6.7
18315	Hanford sandy loam	7.6	21.1	23.9	22.8	178.0	13.3	None
18327	Hanford fine sandy loam	5.1	20.6	24.4	21.1	304.0	18.4	None
18318	Ramona loam	7.0	18.2	27.0	27.3	160.0	48.3	1.1
18310	Yolo loam	8.8	18.5	28.4	29.7	110.0	53.5	4.0
18316	Ramona loam	11.4	16.0	27.0	28.4	40.5	69.0	5.2
18312	Yolo clay loam	5.0	16.1	28.5	29.5	222.0	77.0	3.5
18317	Indio sandy loam	6.7	13.2	23.5	27.6	92.0	78.0	17.4
18320	Chino clay loam	6.4	12.8	27.4	25.4	100.0	114.0	None
18311	Hanford sand	4.2	11.0	23.6	22.6	162.0	115.0	None

* Average dry weight (grams) of tops, per pot.

inorganic phosphate in the stem-sheath material and response to phosphate. Without exception, those plants which grew on soils substantially deficient in phosphate (growth increases ranging from 48 to 115 per cent) were very low in inorganic phosphate, whereas those which grew in soils showing little or no phosphate deficiency contained considerably more phosphate. A set of earlier results with oats in the vegetative stage grown in 11 other soils are recorded in table 4. These results are consistent with those of table 3.

That additions of phosphate to the nutrient medium will result in an increased accumulation of PO_4 in the plant is again shown by the data of table 5. It will be noted that in many cases phosphate applications caused markedly increased amounts of PO_4 in the plants. Since the plants grown on the first

eight soils of this series showed little or no response from phosphate fertilizer, the increases in inorganic phosphate within the plants resulting from phosphate additions represent "luxury" consumption.

It will be noted that applications of potassium sulfate slightly reduced the inorganic phosphate content of the plants when grown in 12 of the 17 soils (compare columns 4 and 5 of table 5). Whether this effect is due to decreased phosphate absorption, as such, or to more complete conversion of inorganic phosphate into organic forms within the plants, cannot be stated. On the other hand, with 4 other soils the application of K_2SO_4 caused the oat plants to accumulate increased amounts of PO_4. Thornton (13) found that potash

TABLE 3

Relationship between response to phosphate applications and the inorganic PO_4 content of green oat plants

(Vegetative stage—plants 26 days old)

SOIL NUMBER	INCREASE IN YIELD FROM PHOSPHATE ADDITIONS	INORGANIC PO_4 IN GREEN OAT PLANTS GROWN IN N-TREATED POTS (STEM-SHEATH MATERIAL 0–1 INCH ABOVE CROWN)
	per cent	*p.p.m.*
18313	None	368.0
18322	None	166.0
18323	0.4	129.0
18327	1.4	334.0
18321	1.5	722.0
18324	1.6	219.0
18325	1.8	306.0
18319	3.1	114.0
18314	11.2	165.0
18315	13.3	64.0
18318	48.3	46.0
18310	53.5	23.0
18316	69.0	53.0
18312	77.0	23.0
18317	78.0	46.0
18320	114.0	46.0
18311	115.0	23.0

fertilization was associated with increased concentrations of inorganic phosphate in corn plants.

Preliminary tests on oat plants at later stages of growth (see table 6) indicated, as in the vegetative stage, that the young growing parts of the oat plant contain higher concentrations of inorganic phosphate than do more mature parts. At the heading stage there is a pronounced gradient in the stem, more phosphate being found in the upper stem and rachis than in the lower parts of the stem. At this, and at the ripening stage, sections of both the lower stem and upper stem were analyzed. The results are recorded in table 7.

It will be noted that there is less correlation between the phosphate responses

and the inorganic phosphate of the plants at maturer stages of growth than at the younger vegetative stages. Although the lower stems of all the plants grown in phosphate-deficient soil were consistently low in inorganic phosphate, a corresponding condition was found in certain of the plants grown in soils that were not deficient in phosphate. Moreover, at the heading stage inorganic phosphate in the upper parts of the stems was in many cases as high where the plants were grown in phosphate-deficient soil as where grown in soil well supplied with phosphate. Apparently, at this stage of growth phosphate is rapidly translocated from the leaves and lower stems to the developing seed. The rate of absorption from the soil probably decreases as maturity approaches, also. However, major differences in the phosphate content of the nutrient media were still reflected in the inorganic phosphate of the plants at the head-

TABLE 4

Relationship between response to phosphate applications and the inorganic PO_4 content of green oat plants

(Vegetative stage—oats 45 days old)

SOIL NUMBER	SOIL TYPE	INCREASE IN YIELD FROM PHOSPHATE ADDITIONS	INORGANIC PO_4 IN GREEN OAT PLANTS GROWN IN N-TREATED POTS (STEM-SHEATH MATERIAL 0–1 INCH ABOVE CROWN)
		per cent	*p.p.m.*
18050	Hanford loam	None	191.0
18048	Placentia loam	14.7	55.0
18051	Hanford sand	48.5	34.0
18067	Kimball sandy loam	121.0	34.0
18066	Yolo clay adobe	131.0	14.0
18057	Yolo clay loam	162.0	13.0
18047	Hanford loam	195.0	11.0
18068	Hanford loam	210.0	11.0
18055	Yolo clay loam	263.0	Trace
18063	Yolo loam	320.0	11.0
18052	Chino clay loam	560.0	Trace

ing stage (N-treated pots as compared with NP-treated pots), but translocation and transformation processes have tended to obscure the more direct relationships noted in early growth stages. That the translocation of PO_4 from the stems to the seeds proceeds very rapidly is indicated by the greatly decreased phosphate concentration in the upper stem at the ripening stage as compared with that found at the early heading stages. In this later stage there is little, if any, correlation between phosphate supply and the inorganic phosphate content of the parts tested.

Effect of nitrogen deficiency upon inorganic phosphate accumulation.—Since all of the soils used in this experiment are more or less deficient in nitrogen, the amounts of inorganic phosphate found in plants grown with and without nitrogen fertilization should show the effect of nitrogen deficiency on the accumulation of inorganic phosphate in the plant. Data bearing on this point are recorded in table 8. They show definitely that nitrogen deficiency causes

inorganic phosphate to accumulate within the plant. In some cases the increases resulting from this cause are very striking. With plants grown in

TABLE 5

Effect of phosphate and potash applications upon inorganic phosphate content of green oat plants
(Vegetative stage—plants 24–26 days old)

SOIL NUMBER	INCREASE IN YIELD FROM PHOSPHATE ADDITIONS	INORGANIC PO₄ IN GREEN OAT PLANTS GROWN IN DIFFERENTIALLY FERTILIZED POTS (STEM-SHEATH MATERIAL 0–1 INCH ABOVE CROWN)		
		N treatment	NP treatment	NPK treatment
	per cent	*p.p.m.*	*p.p.m.*	*p.p.m.*
18313	None	368	890	668
18322	None	166	368	712
18323	0.4	129	503	445
18327	1.4	334	890	813
18321	1.5	722	890	654
18324	1.6	219	709	798
18325	1.8	306	989	797
18319	3.1	114	1,023	829
18314	11.2	165	712	712
18315	13.3	64	844	736
18318	48.3	46	859	668
18310	53.5	23	167	140
18316	69.0	53	973	890
18312	77.0	23	404	380
18317	78.0	46	219	252
18320	114.0	46	320	300
18311	115.0	23	167	184

TABLE 6

Distribution of inorganic phosphate in oat plant at heading stage

PART OF PLANT			INORGANIC PO₄ IN VARIOUS PARTS OF GREEN OAT PLANT	
			Soil 18313	Soil 18327
			p.p.m.	*p.p.m.*
Stem		Between uppermost node and rachis...	406.0	611.0
		2 inches below uppermost node........	170.0	231.0
		4–6 inches above crown..............	....	83.0
		0–2 inches above crown.............	27.0	50.0
Leaves	Upper	Blade....................	....	....
		Sheath....................	184.0	....
	Middle	Blade....................	Medium	Medium
		Sheath....................	....	150.0
	Lower	Blade....................	Low	Low
		Sheath....................	83.0	61.0

phosphate deficient soils, little or no accumulation of inorganic phosphate occurred in the early growth stages, no doubt because the plants at this stage were suffering more or less equally from both nitrogen and phosphate defi-

ciency. At the heading stage, however, the accumulation of PO_4 was more evident.

Gilbert, McLean, and Adams (6), Thornton (13), and McCool and Weldon (8) have obtained similar results, the first named investigators having found that low nitrate may cause inorganic phosphate to accumulate in the plant, and conversely low phosphate causes the accumulation of nitrate. McCool and Weldon (8) state: "If one element is decidedly a limiting factor, the slow growth of the plant appears to permit the accumulation of high concentrations of other elements."

TABLE 7

Relationship between response to phosphate applications and the inorganic PO_4 in green oat plants

SOIL NUMBER	INCREASE IN YIELD FROM PHOSPHATE ADDITIONS	INORGANIC PO₄ IN STEMS OF OAT PLANT					
		Heading stage*				Ripening stage†	
		Fertilized with N only		Fertilized with NP		Fertilized with N only	
		Lower stem 0–2 inches above crown	Upper stem 0–4 inches below rachis	Lower stem 0–2 inches above crown	Upper stem 0–4 inches below rachis	Lower stem 0–2 inches above crown	Upper stem 0–4 inches below rachis
	per cent	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
18131	None	22.0	695.0	1,242.0	973.0	46.0	76.0
18322	None	25.0	532.0	....	....	30.0	46.0
18323	0.4	164.0	813.0	....	....	Trace	46.0
18327	1.4	50.0	813.0	....	....	....	....
18321	1.5	1,149.0	1,149.0	....	....	1,110.0	276.0
18324	1.6	32.0	709.0	....	....	....	....
18325	1.8	164.0	813.0	....	....	....	....
18319	3.1	25.0	668.0	....	....	30.0	46.0
18314	11.2	27.0	641.0	....	....	30.0	107.0
18315	13.3	61.0	627.0	....	....	41.0	107.0
18318	48.3	25.0	586.0	....	....	30.0	76.0
18310	53.5	27.0	709.0	46.0	844.0	22.0	76.0
18316	69.0	22.0	371.0	1,352.0	1,023.0	25.0	46.0
18312	77.0	22.0	411.0	682.0	813.0	42.0	46.0
18317	78.0	15.0	166.0	....	....	Trace	37.0
18320	114.0	25.0	767.0	....	....	Trace	41.0
18311	115.0	27.0	133.0	219.0	813.0	46.0	46.0

* Heading stage, plants 44 days old.
† Ripening stage, plants 66 days old.

It has often been shown that limitation of growth or metabolism by any one of various factors may cause an increase or decrease of various constituents within the plant. The writer has found that the leaves and woody stems of citrus affected with "mottle leaf" contain inorganic phosphate greatly in excess of that in corresponding healthy leaves and branches. It is reasonably certain, however, that this accumulation of inorganic phosphate is not the primary cause of mottle leaf but rather the result of arrested growth and disturbed metabolism.

Since inorganic phosphate may accumulate in plants as a result of nitrogen deficiency, certain physiological diseases, and probably other adverse conditions affecting growth or metabolism, it is apparent that an important limitation is imposed on the use of this method in the diagnosis of phosphate deficiencies. In other words, it will probably be impossible to determine from a test made under sub-optimum conditions with respect to one or more other growth factors whether or not supplemental phosphate will be required when these sub-optimum factors have been corrected. All that may be reasonably hoped for is that the method may indicate *under the particular complex of conditions which actually prevail* whether or not the plant is securing adequate

TABLE 8

Effect of nitrogen deficiency upon the accumulation of inorganic phosphate in green oat plants

SOIL NUMBER	VEGETATIVE STAGE				HEADING STAGE			
	Response to nitrogen	Response to phosphate	Inorganic PO₄ in lower stems		Response to nitrogen	Response to phosphate	Inorganic PO₄ in lower stems	
			Without N	With N			Without N	With N
			p.p.m.	*p.p.m.*			*p.p.m.*	*p.p.m.*
18325	Strong	None	829.0	300.0	....	....	....	.....
18327	Medium	None	1,149.0	334.0	....	....	....	.....
18324	Medium	None	940.0	219.0	....	....	....	.....
18323	Medium	None	709.0	129.0	....	....	....	.....
18313	Medium	None	712.0	368.0	Strong +	None	1,242.0	22.0
18314	Slight	None	528.0	165.0	....	....	....	.....
18315	Slight	None	116.0	64.0	....	....	....	.....
18321	Very slight	None	1,056.0	722.0	....	....	....	.....
18312	Slight	Slight	41.0	23.0	Strong	Medium	259.0	22.0
18320	Slight	Medium	46.0	46.0	Strong	Strong	57.0	25.0
18318	Slight	Slight	46.0	46.0	Medium	Slight +	167.0	25.0
18310	None	Slight	23.0	23.0	Medium +	Medium +	151.0	27.0
18316	None	Slight	35.0	53.0	Slight +	Medium	169.0	22.0
18311	Slight	Medium	41.0	23.0	Slight	Strong	46.0	27.0
18317	Very slight	Medium	46.0	46.0	Slight	Strong	45.0	15.0

phosphate. However, any method that will give accurate results even under this condition will obviously prove exceedingly useful.

Results with lemon plants

Some preliminary data obtained with Lisbon lemon cuttings grown for several months in complete and in phosphate-deficient culture solutions[3] are in harmony with those on the young oat plants. The amounts of inorganic phosphate found in various parts of the citrus plants, grown under the conditions mentioned, are shown in table 9. Except in the young immature leaves, no part of the lemon plants that had been grown from 4 to 7 months in

[3] These lemon cuttings were furnished through the kindness of Dr. A. R. C. Haas.

phosphate-deficient solutions contained more than a trace of inorganic phosphate, and even in the very young developing terminal leaves the amount found was too low to be determined by the method used. These small immature leaves, however, eventually develop into full-sized mature leaves; this is accomplished presumably at the expense of phosphate derived from older parts of the plant. This observation suggests that for leaf growth in the lemon only a very small quantity of inorganic phosphate is needed at any one time provided the supply is adequately maintained.

TABLE 9

Inorganic PO₄ content of lemon cuttings grown in culture solutions

CULTURE SOLUTION	PART OF PLANT	INORGANIC PO_4 OF GREEN TISSUE
		p.p.m.
Complete	Terminal leaf (immature)	133.0
	5th leaf from terminal (immature)	133.0
	10th leaf from terminal (mature)	104.0
	Original leaf of cutting	133.0
	Woody tissue from original cutting	735.0
Moderately deficient in phosphate	Terminal leaf (immature)	Low
	5th leaf from terminal	Trace
	9th leaf from terminal	Trace
	Original leaf from cutting	Low
(Plants grown 4 months)	Woody tissue from original cutting	Trace
Extremely deficient in phosphate	Terminal leaf (immature)	Low
	4th leaf from terminal	Trace
	8th leaf from terminal	None detected
	Original leaf from cutting	Trace
(Plants grown 7 months)	Terminal stem	Low
	Stem 10 inches above base	Trace
	Stem 1 inch above base	Trace
	Stem original cutting	Trace
	Old roots	Trace

In contrast to the low concentration of inorganic phosphate found in the plants grown in phosphate-deficient solutions, all parts of the cuttings grown in complete nutrient solution were relatively high in inorganic phosphate. The woody conductive tissue was especially high.

DISCUSSION

The data obtained with oats clearly indicate that at certain growth stages there is a striking relationship between the inorganic phosphate of green plant

tissue and the nutrient supply. At later growth stages this relationship is less apparent; translocation and transformation changes coupled, perhaps, with a less rapid rate of absorption are probably the factors chiefly responsible. Although the concentration of inorganic phosphate in different parts of the same plant is variable, being highest usually in conductive, embryonic, and storage tissues, all parts appear to be consistent at early growth stages in reflecting the variable supply in the nutrient medium.

It has been shown, however, that if growth is more limited by some other factor than by phosphate, inorganic phosphate may accumulate within the plant. Hence, it will probably be impossible to predict from a test on a given plant grown under sub-optimum conditions with respect to other growth factors whether the phosphate supply would be ample when these other sub-optimum conditions have been corrected. All that may reasonably be expected is that the plant will indicate, *under the particular complex of conditions in which it is growing*, whether or not the phosphate supply is ample.

SUMMARY

Tests for inorganic phosphate in oat plants grown in a series of soils, and in citrus plants grown in culture solutions, have shown the following:

In the vegetative stages, oats limited in growth by insufficient phosphate were exceedingly low in inorganic phosphate; this was true of all parts of the plant. Where not limited by phosphate much higher quantities were found in the green plant. This same condition was found in Lisbon lemon cuttings grown in culture solutions.

Applications of phosphate fertilizer greatly increased the absorption of phosphate by oat plants; all parts of the plant showed this effect in the vegetative stages, the embryonic, conductive, and storage tissues being most markedly affected.

As a result of translocation and transformation changes within the oat plant at later growth stages, the aforementioned relations were not nearly so pronounced. Less rapid absorption of phosphate at these stages may also have accounted in part for the lack of correlation.

Nitrogen deficiencies caused inorganic phosphate to accumulate within the oat plant, even in soils which were deficient in phosphate.

Marked accumulations of inorganic phosphate have been found in the leaves and stems of citrus branches affected with the physiological disease known as "mottle leaf." It is probable that physiological disturbances produced by various other conditions may also cause inorganic phosphate to accumulate within the plant.

Although subject to the limitations noted in the foregoing, the method, if worked out in sufficient detail for different plants, may prove very useful.

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CARBOHYDRATE SUPPLY AS A PRIMARY FACTOR IN LEGUME SYMBIOSIS

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The necessity for a supply of carbohydrate in legume symbiosis has long been recognized, but the more recent experimental evidence in this and closely related fields has added greatly to our knowledge of the subject. An attempt is made here to present and correlate some of the more pertinent facts, gathered from the literature and from studies carried out in this laboratory, with the view to showing in greater detail the manner in which the bacteria and host respond to varying rates of photosynthesis.

These studies seem to show that the available carbohydrate supply (chiefly sugars and starch) is a primary factor in determining nodule location, growth, and size; harmful effect of fixed nitrogen on nodulation; quantity of nitrogen fixed by good strains; disintegration of nodules; and other similar phenomena. It is desired especially to emphasize that this discussion, unless otherwise stated, deals with plants growing under *normal soil conditions where nitrogen is rather deficient.* Certainly under many other conditions, such as high acidity, deficient calcium, and insufficient moisture [*see* excellent monograph by Fred Baldwin, and McCoy (14)], other factors may outweigh carbohydrate supply in importance. It should also be stressed that this paper deals primarily with symbiosis involving *good nitrogen-fixing bacterial strains,* and is not concerned with the reasons for variations in infectiveness (ability to produce nodules) or effectiveness (ability to supply the host plant with nitrogen) of different strains. Very little is known about the reasons for these variations, and whether the carbohydrate supply is a factor of major importance remains to be determined.

CARBOHYDRATE SUPPLY AND NODULE FORMATION

A supply of carbohydrate is needed for every step in nodule formation, but the quantity required varies in the different stages of development.

Traces of organic matter are normally liberated from the roots of plants in the forms of sloughed-off root cells and possibly also as excretions. It is

probable that this food supply stimulates the growth of legume bacteria present. A high carbohydrate concentration in the root is not a prerequisite for bacterial entrance.

Why do nodule bacteria enter roots? It has been shown that they will enter and form nodules on roots growing in (a) dilute mineral solutions, (b) sugar solutions, (c) weak nitrate solutions, and (d) complete nutrient media. This indicates that they do not penetrate roots for any very specific reason. They seem to grow wherever the growth conditions and food supply are satisfactory, whether outside or inside the host cell.

Several interesting phases of bacterial entrance, not yet worked out in complete detail, have been considered by McCoy (31) and by Thornton (53). The nature of the infection thread is described fully by Thornton (52) and the development of the nodule by Thornton (53) and by Brenchley and Thornton (7).

It is at the time of the growth of nodule tissue that the carbohydrate supply becomes of major importance. The penetration of the infection thread stimulates rapid cell division and growth; likewise, there occurs a great increase in bacterial numbers. Very shortly thereafter nitrogen fixation begins, and a marked growth of the host tissue follows. All of the activities of the plant are suddenly accelerated and these require an ever increasing supply of carbohydrate.

It should be borne in mind that the sugar synthesized in the leaves, moves to other parts of the plant by a process somewhat similar to, but more rapid than, diffusion. However, during active photosynthesis, it commonly accumulates to a considerable extent in the tops, as shown by Mason and Maskell (32) in the cotton plant. Miller (33) mentions several experiments with both legumes and nonlegumes showing that there is an osmotic pressure gradient in plants. This gradient is undoubtedly a factor of considerable importance in legume nodulation.

CARBOHYDRATE UTILIZATION AS A FUNCTION OF AMOUNT SYNTHESIZED

Since good nodule development depends upon an abundant carbohydrate supply, it is proposed to show what use the plant makes of the photosynthetic carbohydrate as the amount formed in the leaves varies from very little to maximum. The facts are shown diagrammatically in figure 1.

This figure is a composite picture for various species of legumes, drawn from bits of information gathered chiefly from the many articles referred to in this paper. It shows the manner in which the carbohydrates are utilized by legumes, growing in an inoculated nitrogen-deficient soil under conditions such that the rate of photosynthesis varies. As is well known, many factors, particularly light intensity, carbon dioxide supply, chlorophyll content, and temperature, greatly affect carbohydrate synthesis. Data showing the weights of the tops and roots of various legumes grown under widely different conditions are rather abundant; similar data giving nodule weights are less abundant but adequate for the present purpose. The respiration data, considered quanti-

tatively, are the least satisfactory, but qualitatively they bear out the ideas
conveyed in figure 1. Emphasis should not be placed upon the details of the
curves but rather upon the broad, general relationships of amount of carbo-
hydrate synthesized in the leaf to respiration, growth, and nodulation.

Before the figure is discussed in detail it should be emphasized that during
the active growth of a plant a fairly wide variation in the rate of photosynthesis
does not necessarily produce a corresponding wide variation in the concentra-
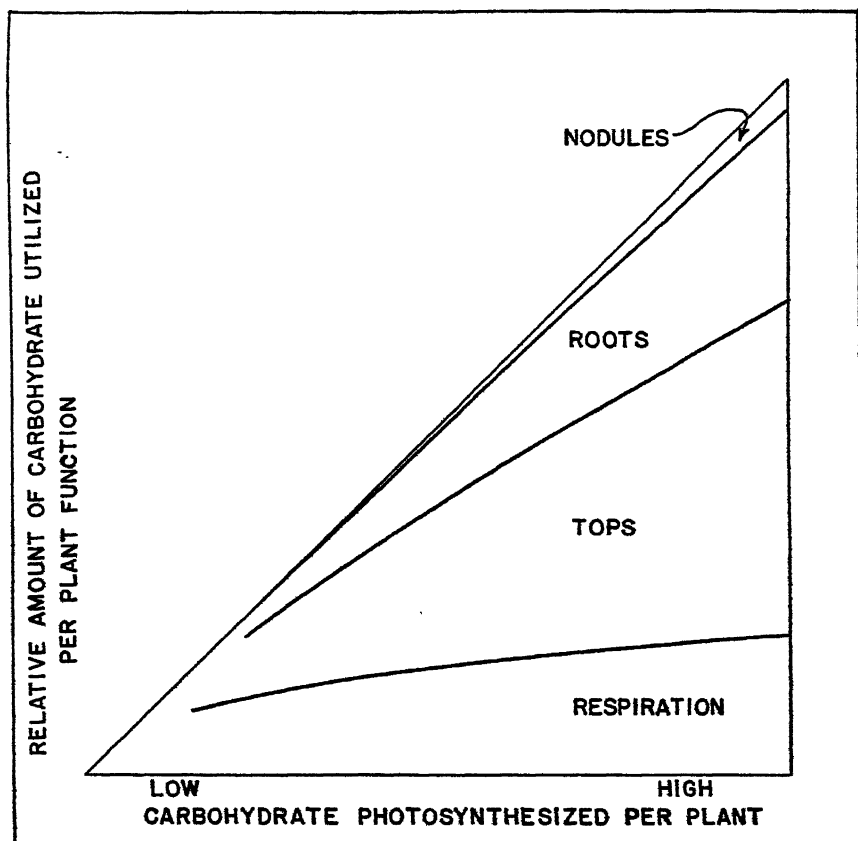
FIG. 1. CARBOHYDRATE UTILIZATION IN LEGUMES AS A FUNCTION OF AMOUNT SYNTHESIZED

tion of soluble carbohydrates in the plant juice, assuming that conditions for
growth are satisfactory (3). The increased sugar, above the needs for respira-
tion, is used in the growth of new plant tissues, or is stored largely as starch.
The data of Arthur, Guthrie, and Newell (5), a small portion of which is shown
in table 1, well illustrate this point. Although the sugars, total available car-
bohydrate, and the carbohydrate-nitrogen ratio usually increased with in-
creased light and carbon dioxide, it was the dry weight of the plant which
showed the greatest increases.

Where photosynthesis is proceeding at a very slow rate it will be noted from figure 1 that the carbohydrate is consumed largely or wholly in respiration.

TABLE 1

Chemical composition of the aerial portion of legumes grown under different environmental conditions

TREATMENT	WEIGHT PER PLANT	TOTAL NITRO-GEN	CARBOHYDRATE—PER CENT DRY WEIGHT				CARBOHYDRATE NITROGEN
			Acid hydro-lyzable	Sucrose	Dextrose	Total	
	gm.	*per cent*					
Red clover, 1925. Age 76 days							
Control.....................	1.1	3.44	9.61	2.28	1.86	13.75	4.0
Greenhouse 1*...............	6.0	2.73	11.80	2.50	2.66	16.96	6.4
Greenhouse 2*...............	9.2	1.96	16.36	2.99	2.70	22.05	11.2
Red clover, 1926. Age 71 days							
Control.....................	2.1	3.18	12.25	2.04	3.52	17.81	5.6
Greenhouse 1*...............	2.2	2.88	11.49	1.93	4.12	17.54	6.0
Greenhouse 2*...............	3.9	1.76	19.14	2.30	5.84	27.28	15.6
Red clover, 1927. Age 66 days							
Control.....................	0.6	2.90	4.92	2.30	1.44	8.66	3.0
Greenhouse 2*...............	5.9	2.13	15.53	4.86	4.58	24.97	11.6
Soybeans, 1925. Age 40 days							
Mandarin, control†...........	7.8	3.71	19.22	2.06	1.38	22.7	6.1
Mandarin, G.H. 1†...........	35.8	2.97	19.33	2.00	2.69	24.0	8.1
Mandarin, G.H. 2†...........	32.1	1.81	26.12	1.81	3.36	31.3	17.2
Peking, control†..............	3.8	4.12	15.03	2.06	0.76	17.9	4.4
Peking, G.H. 1...............	19.5	3.32	15.97	2.10	1.38	19.5	5.9
Peking, G.H. 2...............	38.1	2.19	19.54	2.35	3.59	25.4	11.6
Tokio, control†...............	11.6	3.73	13.63	1.66	0.81	17.1	4.6
Tokio, G.H. 1................	30.8	3.79	15.22	2.37	0.68	18.3	4.9
Tokio, G.H. 2................	52.1	2.57	16.75	2.57	3.92	23.2	9.1
Biloxi, control†..............	14.2	3.81	16.40	1.72	0.91	19.0	5.0
Biloxi, G.H. 1................	52.8	3.19	14.86	2.54	1.63	19.0	6.0
Biloxi, G.H. 2†...............	50.8	1.50	30.84	1.82	2.28	34.9	23.0

Control plants grown in ordinary greenhouse.
Greenhouse 1, greenhouse plus 6 hours artificial light daily.
Greenhouse 2, same as greenhouse 1 plus extra CO_2 (about 0.3 per cent).
Greenhouse 2 (1927), received 12 hours artificial light and extra CO_2.
* Flowering.
† Fruiting or flowering.

With increased photosynthesis a point is soon reached where the supply of carbohydrate is adequate for appreciable growth. Usually considerably more of the carbohydrate is used in the production of leaves and stems than of roots,

but the relative amounts used in the two portions of the plant vary widely with the plant species and growth conditions. No appreciable nodule development takes place until the carbohydrate supply is adequate for a fair amount of root growth, as has been shown experimentally many times (3). It is obvious, then, that practically any set of conditions unfavorable for root growth is at the same time more unfavorable for nodulation.

If the carbohydrate supply is adequate for good nodule formation it will be observed from the figure that additional carbohydrate merely means more tops, roots, and nodules, the increases of each being somewhat regular.

Respiration, which consumes practically all of the carbohydrate where little is synthesized, consumes a smaller and smaller percentage of the total where photosynthesis is increased. The absolute amount consumed in respiration under these conditions constantly increases, of course, because of increased dry matter and somewhat higher sugar concentration, but relatively on a per plant basis a smaller percentage of the total carbohydrate is so burned up.

The rate of photosynthesis at which appreciable nodule growth will occur varies with the legume species and other factors but particularly with the quantity of soluble soil nitrogen. If the supply is very abundant a large percentage of the carbohydrate not required for respiration will go into top growth, leaving little for root growth, even though the rate of photosynthesis is rather high. If, on the other hand, the supply of fixed nitrogen is limited, as specified in connection with figure 1, an abundance of top growth without considerable root growth and fair nodulation is impossible for the obvious reason that nodules must form and nitrogen fixation begin before there is an adequate supply of nitrogen for either top or root growth.

The results of Eaton (12) with soybeans well illustrate an intermediate condition, so far as nitrogen is concerned. Even though a nutrient solution containing small amounts of nitrate nitrogen was added daily, he observed that growth and nodule development were correlated with the length of day, severity of leaf clipping, and percentage of carbohydrates. Certain other correlations would, undoubtedly, have been in evidence if a nitrogen-free medium had been used.

Nodule growth normally lags slightly behind root growth probably partly because of the difficulty of getting sufficient carbohydrate into the nodule rapidly enough. Not only is the base of a nodule usually constricted, but the quantity of carbohydrate consumed per unit of nodule tissue is probably higher than per unit of root tissue.

CARBOHYDRATE ABSORPTION THROUGH THE ROOT

Although emphasis in the foregoing discussion has been placed upon photosynthetic carbohydrate, the fact should not be overlooked that plants can to some extent absorb certain sugars through the roots and utilize them in the same manner as if synthesized in the leaves (33, p. 239–241). If sugar is absorbed in this manner by plants growing in normal light, then both sources of

sugar will be used simultaneously and, since the normal sugar gradient will no longer exist, we should expect an unusually good growth of roots and with little lag in nodule development. The marked increase in root growth following the addition of sugars has been observed frequently. The data of Knudson (23), a portion of which is given in table 2, illustrate this point. Vetch plants, growing in the presence of sucrose, showed a top-root ratio of 1.2 as contrasted with a ratio of 4.6 without sugar.

Several experiments have been reported which show the effect of sugar additions on nodules, for example, Golding (18), Prucha (42), Wilson (57), Leonard (26), and Georgi, Orcutt, and Wilson (16). Wilson (58) and Lewis and McCoy (27) obtained nodules on plant roots kept in sugar solutions in

TABLE 2

Influence of various sugars on the growth of vetch in water cultures under sterile conditions
(Duration of experiment 39 days)

CULTURE SOLUTION	NUM-BER OF PLANTS	DRY WEIGHT OF ROOTS	DRY WEIGHT OF TOPS	TOTAL DRY WEIGHT	AVER-AGE TOTAL DRY WEIGHT PER PLANT	GAIN IN WEIGHT PER PLANT[*]	SUGAR AB-SORBED	TOP/ROOT RATIOS
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
Check (no sugar).............	2	13	67	80	40.0	11.0		5.2
Check (no sugar).............	2	15	68	83	41.5	12.5		4.5
Check (no sugar).............	1	8	32	40	40.0	11.0		4.0
Lactose.....................	1	16	40	56	56.0	27.0	49.0	2.5
Glucose.....................	2	41	81	122	61.0	32.0	72.6	2.0
Glucose.....................	1	30	62	92	92.0	63.0	66.5	2.1
Maltose.....................	1	20	42	62	62.0	33.0	24.4	2.1
Fructose....................	2	39	75	114	57.0	28.0	113.0	1.9
Sucrose..:..................	1	55	60	115	115.0	86.0	133.4	1.1
Sucrose.....................	2	77	96	173	86.5	57.5	141.0	1.2

* The weight of each seed was 29 mgm.

the dark. Hopkins and Fred (21) observed that mannitol partially counteracted the effect of nitrogen additions on nodule production, and Allison and Ludwig (3) obtained a similar effect with sucrose.

RELATIVE CARBOHYDRATE REQUIREMENTS OF THE HOST PLANT AND OF THE BACTERIA

A better understanding of the rôle of carbohydrate in legume symbiosis may be had if consideration is given to the relative needs of the bacteria and host for the carbohydrate synthesized in the leaves. In order to produce 1 gm. of dry weight about 5 to 20 gm. of glucose are required in the case of most aerobic bacteria, whereas only about 1.35 gm. are required for the more efficient higher plants (29, 50, 54).

What are the relative weights of the bacteria in the nodule and of the host

plant? The dry weight of nodules on annuals grown under very exceptionally favorable conditiØns is about 7 per cent of the total dry weight of the whole plant, as shown by Giöbel (17), and by Jones and Tisdale (22). Probably less than 1 per cent of the total weight of the average legume plant represents bacterial cells.

The activity of the bacteria in the nodule is an important consideration. The evidence shows that they are present largely as bacterioids, which are not being digested rapidly (17), and which are mostly incapable of multiplication, but which are almost certainly consuming some energy in respiration. Furthermore, the energy requirements for nitrogen fixation, as is pointed in the following, are probably negligible.

These facts indicate very strongly that the bacteria in the nodules consume a very small proportion of the total carbohydrate photosynthesized.

There is another type of evidence that throws light on the carbohydrate requirement of the host, relative to that of the nodule bacteria. Newton (37) and Reinau (43, 44, 45) have shown that the roots of inoculated legumes give off about twice as much CO_2 as do the roots of nonlegumes. Reinau does not, however, prove satisfactorily that this difference is due to the respiration of the nodule bacteria rather than to the natural differences in the plant species, wholly apart from the bacteria.

Let us, however, assume that (a) the excess respiration is due to the bacteria as Reinau thought, (b) unit weights of root and top tissues (exclusive of bacteroidal tissue) respire at the same rate, (c) the legume roots represent 30 per cent of the total weight of the plant, and (d) legumes use 25 per cent of the photosynthesized carbohydrate in respiration. Under these conditions about 5.8 per cent of the carbohydrate synthesized is used by the nodule bacteria in respiration and nitrogen fixation. According to Lundegårdh (29) the respiration rate of the tops is usually higher than that of the roots. The recently reported data of Müller (36) show that *Sinapis alba* had relative respiration rates of 2.95 and 1.36 for the tops and roots. If we use these figures in the foregoing calculations then only 3.5 per cent of the carbohydrate photosynthesized is used by the bacteria.

Both lines of evidence presented indicate, then, that the bacterial requirements for respiration and nitrogen fixation are probably not greater than 3 to 6 per cent of the total carbohydrate photosynthesized under good growing conditions. This estimate is, admittedly, not very accurate, and is probably high rather than low.

CARBOHYDRATE AND THE LOCATION OF NODULES

Under most conditions nodules are located principally at the tops of the root system on or near the main roots, especially in the case of annuals or during the first year's growth of other legumes. Several reasons have been advanced for this, such as better aeration and immunity. Evidence is presented in the following which seems to show that the carbohydrate supply is a primary

factor in nodule location, assuming uniform inoculation at the time of seeding, and a satisfactory soil environment. Under many conditions, of course, carbohydrate supply would be a secondary factor.

Normal nodules. The experiments of Nobbe, Schmid, Hiltner, and Hotter (39) and of Otis (40) showed that where plants were inoculated at different depths the nodules always formed only at the point of inoculation. Löhnis (28) and Ludwig at this laboratory observed that delayed inoculation resulted in nodules occurring much lower on the root system. Normally not only are most of the nodules located near the soil surface but also near the tap root, whereas other rootlets located just as near the surface but several inches from the larger central roots show few or no nodules. Aeration is apparently a very secondary factor under these conditions. The writer has frequently observed that in the case of soybean seedlings growing in the presence of a heavy inoculation, a single rootlet may have 10 to 20 nodules. At first they are of uniform size, but soon the ones nearest the main root become much larger while those farther away remain small or disappear. The whole tip of the rootlet beyond the nodules frequently appears stunted, almost certainly as a result of under-nourishment.

Nodule development is usually best in the region of the tap root and nearest the source of carbohydrate supply. Löhnis (28) observed that the upper portions of the root system commonly show starch deposits, while the lower roots, especially the root tips, are free from these. Uninoculated plants, however, if growing in a nitrogen-deficient medium, usually show starch deposits in most of the root cells except at the extreme tip. After nodules have formed on the upper roots and are actively fixing nitrogen the abundant supply of nitrogen, originating in the nodule, markedly stimulates growth and utilization of the carbohydrate as fast as formed.

In the experiments already noted, where inoculation was delayed, sufficient carbohydrate was present in the lower portions of the root system to enable nodules to develop. Active photosynthesis and slow growth, due to inadequate nitrogen, must necessarily result in carbohydrate accumulation. The failure of nodules to form on the upper portion of the root system, already well-nodulated at lower depths, may be due either to insufficient carbohydrate or to lack of root hairs.

Nodules in water cultures. Nobbe and Hiltner (38), Hiltner (19), and the writer observed that legumes produced small, inefficient, scattered nodules when grown in nitrogen-free nutrient solutions. When some of the solution was poured off, a strikingly rapid growth took place in the nodules located above the solution, and plant growth increased markedly.

We may, of course, explain this by simply saying that nodules develop in air better than they do in water, but in the opinion of the writer a more plausible and fundamental explanation is one based on the carbohydrate-nitrogen relationships. In the submerged nodules no appreciable quantity of nitrogen was fixed, but photosynthetic conditions were favorable, and nodules could form

everywhere. When a portion of the solution was poured off, a mass of large, normal nodules, active in nitrogen fixation, formed above the liquid, and the carbohydrate supply was then used largely to meet the increased plant and bacterial needs. Even as early as 1891 Laurent (25) observed that pea nodules grown submerged in water cultures contained much starch and few bacteroids and fixed little nitrogen. Nodules formed above the water contained little starch, were packed with bacteroids, and fixed considerable nitrogen.

The writer grew soybeans in a 0.5 per cent sucrose-mineral medium, containing no nitrogen. About 40 small scattered nodules were formed per plant both in the presence and in the absence of sugar. Apparently little or no nitrogen was fixed. In this case the plants grown both with and without sugar were high in carbohydrate, and hence, as expected, added sugar had no effect.

Non-effective nodules. The results of several recent workers have agreed in showing that nodules produced by poor nitrogen fixing strains of bacteria are usually small, rather numerous, and widely scattered over the root system. With ineffective strains the carbohydrate-nitrogen ratio is necessarily wide, starch occurs (28) almost to the root tips, and the nodules occur all over the root system. The reason for the failure of the ineffective nodules to become large or to form in excessively large numbers is not known. The explanation may revolve in part about the strain, but it seems more probable that nitrogen deficiency is the more important factor.

It has recently been shown by Dunham and Baldwin (11) that where plants are first inoculated with a non-effective strain and become well nodulated, additional nodules will form if an effective strain is then added. If the plants are first inoculated with good strains and several days later with poor strains the nodules are due wholly, or nearly so, to the good bacteria. A simultaneous inoculation with good and poor strains results in nodule formation by both. These workers point out that both good and poor strains vary in their ability to inoculate a plant, but one group is not necessarily superior to the other in infectiveness. Any given strain may be made either more or less effective in nitrogen fixation, according to Allen and Baldwin (2) and others, by repeated plant passage.

These findings agree with the idea that nodule formation is closely connected with carbohydrate supply in the root if we make the logical assumption that with ineffective strains only present, the carbohydrate is more plentiful than if effective strains are present. More information is needed, however, before a positive conclusion can be reached.

Nodules growing in a nitrogen-free atmosphere. The results of Kossowitsch (24) and of Whiting (56), particularly the latter, are of unusual significance in the present discussion. In the experiments of Kossowitsch, inoculated pea seedlings placed in a sand medium with the roots in a mixture of oxygen and hydrogen made a negligible growth but formed numerous nodules. Whiting grew inoculated cowpea seedlings with the roots in a gas mixture consisting of 97 per cent oxygen and 3 per cent carbon dioxide. The plants made little

growth but formed many very small nodules scattered over the root system practically to the root tips. Where nitrogen gas was present good fixation occurred and the nodules in this case were very large and located mostly on the upper and older part of the root system.

In these experiments, where the roots received neither free nor fixed nitrogen, the nodules formed were almost identical in appearance and location with those produced by non-effective bacterial strains on plants growing in air. This definitely eliminates the bacterial strain as the sole cause of the location and size of the ineffective type of nodule. Under the experimental conditions of Kossowitsch and of Whiting, as well as in the presence of non-fixing strains, an abundance of carbohydrate accumulates in the roots, and nodules can form all over the root system but they remain small, probably because of insufficient combined nitrogen.

It would seem from the foregoing experiments that nitrogen fixation is somewhat incidental in legume symbiosis, since nodulation occurred even though nitrogen fixation could not take place.

Nodules growing on plants supplied with increased carbon dioxide. Riedels (46) reported that the use of additional CO_2 increased the number of nodules on beans 5-fold and increased plant growth. Wilson, Fred, and Salmon (59) commonly obtained increases of 100 to 200 per cent in dry weights and nitrogen fixation with red clover when the partial pressure of CO_2 was increased from that in normal air to 0.2 or 0.4 per cent. The plants had two or three times as many nodules as the controls in air, and the size was greatly increased. Where the plants were grown in 0.5 per cent CO_2 the nodule distribution was very similar to that of plants inoculated with poor strains of the organisms. The largest nodules were clustered near the crown on the tap root with the small, round variety scattered among the secondary roots.

These data emphasize the important rôle that carbohydrate supply plays in nodule location. Under conditions of increased CO_2 the rate of photosynthesis was so rapid that even though the nodules were actively fixing nitrogen there was adequate carbohydrate to allow small nodules to form on the secondary roots, just as occurs with non-effective strains where carbohydrates accumulate because of lack of nitrogen.

CARBOHYDRATE SUPPLY AND THE PHYSIOLOGICAL CONDITION OF THE NODULE

The condition of the nodule is also dependent upon the carbohydrate supply. An attempt is made in the following to present the evidence for this statement, and in so doing special reference is made to certain instances of abnormal nodules.

Normal nodules. A description of the nodule from broad bean (*Vicia faba*), as reported by Brenchley and Thornton (7), may be considered as rather typical. Usually some starch is present, especially near the base of the nodule, but not in excessive amounts. Very starchy nodules are likely to be rather ineffective and, of course, contain fewer bacteria, as Peirce (41) pointed out

many years ago. In normal nodules little or no starch is found in the apex of the nodule where the meristem tissue is located. The proper functioning of the nodule seems to demand that a readily available carbohydrate supply be maintained.

Bean nodules. Bean nodules are of interest because they commonly, or at least frequently, fix little or no nitrogen. They would, therefore, if the ideas presented here are correct, be expected to contain an abundance of starch under conditions of normal photosynthesis.

Deposition of starch, according to McCoy (30), begins early in the development of these nodules, starch grains being recognizable in a section of an infection spot so young as to have spread only part way across the root cortex. She states that there is decidedly more starch in the average bean nodule than in the normal nodules of other plants. McCoy was of the opinion that starch grains once formed are inaccessible to the bacteria so long as they are surrounded by the bodies of the plastids. If this is the case then the condition in

TABLE 3

The effect of darkening or removing tops on the starch content of young pea nodules

	STARCH REACTION AFTER 3 DAYS—PER CENT				
	0	Trace	Weak	Medium	Strong
Group A—Plants in light.................	0	0	16	40	44
Group B—Plants darkened...............	7	36	37	18	2
Group C—Plants with tops removed......	12	10	31	40	7
	STARCH REACTION AFTER 5 DAYS—PER CENT				
Group A—Plants in light.................	0	2	14	34	50
Group B—Plants darkened...............	19	55	16	5	3
Group C—Plants with tops removed......	3	31	38	28	0

these nodules must be somewhat abnormal or pathological. In nodules on other species of legumes the starch is quickly used by the host and bacteria when the plants are darkened or other methods used to cut off photosynthesis. The results of Rippel and Poschenrieder (47), summarized in table 3, illustrate this point. According to Löhnis (28) neither effective nor ineffective strains of nodule bacteria can utilize starch. The cells of higher plants can, however, do this readily (33) under normal conditions.

Boron-deficient nodules. The necessity for an adequate supply of carbohydrate is well-illustrated in the case of broad beans grown in the absence of boron. Brenchley and Thornton (7) observed that in such plants the early development of the nodule is normal whereas the later development depends largely upon the extent to which vascular strands are formed. In the absence of boron frequently no strands develop.

In these boron-deficient nodules the infection thread, containing rod forms, undergoes a remarkable development and finally breaks up the nodule tissue.

The bacteria usually remain as rods and cocci and may become actively parasitic and destroy the protoplasm of the host cell early in the life of the nodule. Where there is a partial development of vascular strands there is a partial development of bacteroidal tissue and the behavior of the bacteria is intermediate. These investigators logically concluded that the bacteria prefer to utilize the carbohydrate supply furnished by the plant through the vascular strands, but if this is inadequate they may under certain conditions use the protoplasm of the host cell.

Disintegrating nodules. According to Thornton (53) three conditions are known under which a good strain of the nodule organism may become parasitic and destroy the tissues of the host. These are (a) where plants such as the broad bean are grown in a boron-deficient medium, (b) where the host plant is put in the dark, and (c) in old nodules. In all cases the lack of an adequate carbohydrate supply is, apparently, the primary reason for the parasitism. Even as early as 1890 Frank (13) pointed out that starch disappears as the nodule starts to disintegrate.

Disintegration of both old nodules and nodules in the dark, according to Thornton, is started by coccoid rods, usually found in the residual pieces of infection thread. These bacteria multiply and remain as rods as they invade the cell walls, finally causing them to collapse. The conditions are similar in the boron-deficient nodules, as already pointed out. The observations of Milovidov (34) were essentially in agreement.

What prevents the disintegration of normal nodules? Thornton (53), as well as others before him, brought up the question of why bacteria growing in normal nodules do not increase up to the limit set by the total energy supply, consuming both the carbohydrates brought to them and also the host tissues. He, as well as a few earlier workers, suggested that the air supply may be the limiting factor. Recent researches throw new light on this question and indicate that a number of factors may be involved.

The organisms exist in the young infection threads as actively growing rods. The tip and younger portions of the infection thread ordinarily consist of irregular zoogloeal masses of bacteria which are largely released into the host cells. These soon cease to divide and are converted into bacteroid forms, which, according to Müller and Stapp (35), Almon (4), Schaede (49), and others, are incapable of multiplication. The active rod forms are limited (52) largely or wholly to the infection threads and to the region just behind the meristem cap where continuous infection of the new cells takes place. Traces of old infection threads with a few rods inside are commonly found scattered throughout normal bacteroidal tissue. This older infection thread, or sheath, according to Thornton (52) and McCoy (31), is continuous with the cell walls of the host plant. McCoy made the very important observation that there is no sheath around the infection thread as it crosses the middle lamella of the cell wall. This indicates that the sheath is deposited by the plant cell as a defense mechanism against the bacterial invasion.

The facts, then, seem to indicate very strongly that in a normal nodule the bacteria are able to live and multiply normally only if protected from the cell contents by the infection thread, whether it be slime-like in nature or resembling a cell wall. If they are liberated into the host cell and lose their protective covering they are ordinarily soon converted into bacteroids. It is not known whether the environment inside a living cell is normally unfavorable for bacterial growth, whether the host secretes something to inactivate the bacteria, or whether the end products of bacterial action, including carbon dioxide, make the environment unsuitable. At any rate it scarcely seems that the oxygen supply is the primary limiting factor, since the bacteria inside these same nodules may grow rapidly if protected by the young infection thread. Furthermore, Barthel (6) and Allison and Hoover (unpublished data) have shown that legume bacteria make good growths in nitrogen-oxygen mixtures of about 0.50 to 2 per cent oxygen. It is improbable that the oxygen concentration inside the nodule would ordinarily be as low as these percentages.

A study of why the bacteria in a normal nodule do not destroy the host tissue limits itself, then, if the foregoing facts are correct, to a consideration of the behavior of the few active rod forms (capable of reproduction) found there, namely, those protected by the infection thread. The indications are that these bacteria do not ordinarily multiply rapidly and hence need little carbohydrate. If these rod forms do multiply rapidly in the presence of available carbohydrate they normally pass out into the cell and are converted into bacteroids. Such continued rapid bacteroid formation is, of course, impossible for purely physical reasons, if for no other. The host cells are soon filled to capacity and the bacteroids are digested slowly, if at all. The situation is obviously quite different from that in a nodule lacking in carbohydrate where the bacteria attack the middle lamella of the cell wall (53) to get energy and, having broken this down, the whole cell collapses.

Although the observations considered in the foregoing clearly point to the necessity for an adequate supply of carbohydrate in order for a nodule to remain healthy, it must be admitted that the available information is still somewhat limited. Much of the discussion revolves around the idea that the nodule bacteria can under certain conditions attack host tissues. Further studies of this phenomenon in particular are needed.

DECREASED NODULE FORMATION IN THE PRESENCE OF COMBINED NITROGEN

A consideration of legume symbiosis from the carbohydrate standpoint allows us to explain the harmful effect of high concentrations of soluble nitrogenous compounds on nodule formation. This subject is considered at length by Allison and Ludwig (3).

If a plant is grown in the presence of an abundance of fixed nitrogen an unusually large percentage of the carbohydrate synthesized is used for top growth and a proportionately smaller root system necessarily develops (15). Since the conditions are usually unfavorable for good root growth we could not

expect nodules to develop normally under these conditions. With moderate nitrate applications nodule tissue may form, but under the same conditions considerable root tissue also develops, the extent of development of both depending primarily upon the root carbohydrate supply, if other growth conditions are satisfactory. The bacteria normally remain alive and ready to produce nodules whenever the carbohydrate supply is adequate, not adequate primarily for the bacteria but for the growth of the tissue of the higher plant in which the bacteria will later live.

It is well to point out the close similarity, so far as nodule development is concerned, between conditions of (a) normal light intensity and high soluble soil nitrogen, and (b) low light intensity and normal soil nitrogen. Under both conditions the available root carbohydrate is low, and growth in this region is limited.

CARBOHYDRATE SUPPLY AND NITROGEN FIXATION

A high rate of photosynthesis usually results in a high rate of nitrogen fixation. Plant growth, nodule development, and nitrogen fixation usually closely parallel one another, as many workers (14) have shown. Almost any condition which favors increased plant growth, other than the application of large quantities of nitrogenous fertilizers, also favors increased activity in the nodule and the formation of new nodule tissue.

There is one particularly outstanding case, however, where nitrogen fixation is negligible even when growing conditions are good and effective bacteria are present. Sometimes seedlings fail to start active nitrogen fixation for several days or weeks, even though kept under excellent light conditions and inoculated with good strains. Nodules will form in the usual period but frequently remain small, and the plant shows no benefit from them. Hiltner and Störmer (20), Rüffer (48), Weber (55), as well as others have observed and discussed this hunger condition. Allam (1) and Rüffer (48) found that in the case of soybeans the nitrogen hunger period was not as much in evidence during the fall as during the summer. Several investigators, for example Fred and Graul (15), have mentioned the favorable effect of small amounts of nitrogen in overcoming this condition. The lag period may last as long as 1 or 2 months in extreme cases, after which nitrogen fixation frequently proceeds at an unusually rapid rate (48) accompanied by the growth of considerable new nodule tissue. The exact cause of the hunger condition is not known with certainty; although excess carbohydrate undoubtedly plays an important rôle, other factors seem to be involved.

ENERGY REQUIREMENTS FOR NITROGEN FIXATION

The literature dealing with legume symbiosis is full of statements or implications to the effect that large quantities of energy are consumed by legume bacteria in the process of nitrogen fixation. Such statements have never been proved and are, in fact, very probably not true. The confusion that seems to

exist is probably due largely to a lack of uniformity of viewpoint in defining the term "energy requirements for nitrogen fixation."

Theoretically, from the strictly chemical standpoint, what are these requirements? The answer to this question can not be given exactly because the chemical steps in the process of nitrogen fixation are unknown. The order of magnitude of these requirements is, however, well known. They are, at most, very small, assuming 100 per cent efficiency of the process; in fact, the nitrogen fixation step may be exothermic and yield energy. Burk (8) points out, for example, that it would be possible for nitrogen to react with carbohydrate to form an alpha-amino acid, $C_6H_{12}O_6N_2$, which process is exothermic. If we assume that the free nitrogen is reduced to ammonia, corresponding to a proteinaceous form, then a definite but small amount of energy is required, since the necessary hydrogen must be derived from organic compounds of carbon. The maximum amount of energy necessary in this case under ideal conditions, according to Burk, corresponds to 1.3 gm. of glucose per gram of nitrogen fixed as ammonia or protein.

Burk determined that the energy requirements for nitrogen fixation, apart from growth, are negligible in the case of *Azotobacter*. Although the apparent energy needs were often large, he found that this was due to an unnecessary consumption of carbohydrate's occurring simultaneously with, but independently of, fixation.

Nitrogen fixation in legumes seems to be a comparable case, the energy requirements for fixation sometimes appearing to be large. When such is the case the apparent energy requirements can almost invariably be accounted for on purely physiological grounds that have no connection with the energy consumed in the chemical process itself, apart from growth.

The definition of the term "energy requirements for nitrogen fixation," then, as used here, is that energy consumed only in the chemical process of fixation and excludes that consumed in the respiration and growth of the bacteria and host. If large quantities of energy are needed for fixation it can only be because the efficiency of the chemical process of fixation is extremely low.

The results of Christiansen-Weniger (10) led him to conclude that the energy requirements for fixation are small. These conclusions were based on experiments with legumes grown with free and fixed nitrogen, exposed to different light intensities, with the calculations based upon the differences in dry weights and nitrogen fixed. The maximum energy requirements for fixation in two experiments were not greater than 5.6 and 7.2 gm. dry matter (22.4 and 28.9 Cals.), respectively, per gram of nitrogen fixed. This includes the requirements for bacterial growth and respiration, which he believed were at least as large as these figures. There were other good physiological reasons for believing these values were too high. Although these data are not free from criticism, Christiansen-Weniger was apparently well aware of most of their shortcomings, and his conclusions seem justified.

Recently Allam (1) concluded from experiments somewhat similar to those

of Christiansen-Weniger that the bacteria required 26.0 and 12.6 gm. of dry matter (104.0 and 50.4 Cals.), respectively, to fix 1 gm. of nitrogen in two experiments. Since his experiments were carried out under conditions of such deficient light that growth and nitrogen fixation were abnormal, the data furnish little information as to the energy requirements for chemical fixation apart from respiration and growth.

The following factors, to a large extent uncontrollable, enter into the final results of such experimentation as reported by Christiansen-Weniger and Allam:

(a) The amount of nitrogen available for the growth of a plant affects the leaf surface and, in turn, photosynthesis. The higher the nitrogen content, usually the greater is the leaf surface per unit dry weight. For instance, Müller (36) found in studies with *Sinapis alba* that plants receiving adequate nitrogen had a leaf area of 126 sq. cm. per gram of total dry substance while similar plants undergoing nitrogen hunger had a leaf area of only 52.8 sq. cm.

(b) It is impossible to keep the rates of growth of plants fed free and fixed nitrogen the same, the latter usually being ahead, which means that the total carbohydrate photosynthesized is greater in this case.

(c) Plants given an adequate supply of fixed nitrogen sometimes have a slightly wider top-root ratio than do nodule-fed plants, with a larger proportion of the dry matter in the form of leaves actively building up carbohydrate. With ordinary quantities of nitrogen the effect on the top-root ratio would be small.

(d) The carbohydrate level in plants fixing nitrogen is commonly slightly higher than in plants given adequate fixed nitrogen. This is evidenced by the slightly lower percentage of nitrogen (wider carbohydrate-nitrogen ratio) usually found in plants living on atmospheric nitrogen. Nodules do not even form until sufficient carbohydrate has accumulated to allow at least a fair root growth.

(e) The amount of nitrogen in a plant affects the chlorophyll content of the leaves, which, in turn, affects the rate of photosynthesis somewhat. Any tendency toward a hunger condition in nodule-bearing plants would ordinarily result in a slightly lower chlorophyll content. Müller (36) observed that the assimilating intensity of leaves from plants receiving insufficient nitrogen was about half as great as from those supplied with plenty of nitrogen under conditions of abundant light; as the light intensity decreased the differences also decreased rather uniformly.

(f) The nodule bacteria require energy for respiration, apart from any that may be needed for fixation, and it is difficult to separate the two requirements experimentally.

(g) It has been claimed by Rippel and Poschenrieder (47) and by others that if considerable energy is required for nitrogen fixation the plant might, presumably, photosynthesize faster and replace the depleted supply. There is little to indicate that such is the case. To the contrary, legumes supplied with fixed nitrogen, as pointed out under (d), are more likely to grow at a slightly lower carbohydrate level than do the nodule-bearing legumes. If plants have the ability to speed up photosynthesis would they not do so in the plant having the lowest amount of carbohydrate, in the one receiving combined nitrogen, rather than in the one fixing nitrogen?

(h) Ammonium nitrate was used as the nitrogen source by Christiansen-Weniger and by Allam, and theoretically 1.3 gm. of sugar is required at 100 per cent efficiency to reduce 1 gm. of nitrate nitrogen to ammonia. According to Burk (9) the efficiency of nitrate reduction is probably not lower than about 32 per cent. About 4 gm. of sugar would then be required to reduce 1 gm. of nitrate nitrogen to ammonia, or 2 gm. to convert 1 gm. of ammonium nitrate nitrogen to ammonia. This affects the final dry weights of the plants to the extent of, at most, only about 4 per cent.

A number of other points of lesser importance might be mentioned, but certainly these are adequate to show that it is practically impossible to determine accurately the energy requirements in nitrogen fixation by the methods previously used. Of the foregoing eight points the first six and probably seven would ordinarily tend to make the final result show a considerable apparent energy requirement for fixation. It should be emphasized, however, that the results of Christiansen-Weniger show a very small maximum requirement, regardless of the points mentioned, and this is exceedingly strong evidence that the requirements for chemical fixation are negligible.

In the foregoing, calculations based on the findings of Newton (37) and of Reinau (45) indicated that the energy requirements of the nodule bacteria for both respiration and nitrogen fixation are probably not greater than about 3 to 6 per cent of the total carbohydrates photosynthesized. These data also indicate that the energy requirements for nitrogen fixation are small.

BACTERIAL-HOST RELATIONSHIP

Many workers in the past have considered that perhaps a type of physiological balance exists between legume nodule bacteria and their hosts under normal conditions. The Hiltner (19) and Suchting (51) immunity theories, for example, were advanced, but these no longer seem plausible. Wunschick (60) and others [see review by Allen and Baldwin (2)] have more recently suggested that there is an equilibrium between the vegetative energy of the higher plant and that of the bacteria.

The newer evidence, together with the old, tends to place much greater emphasis on carbohydrate nutrition in so far as effective bacterial strains are concerned. If the carbohydrate supply is adequate, nodules usually develop and nitrogen fixation roughly parallels the growth of the higher plant; if the supply then becomes deficient, the bacteria sometimes remain more or less dormant in the nodule, or in other cases may attack the tissues of the host to obtain food. As has been pointed out, many factors other than carbohydrate, such as calcium deficiency, high acidity, and insufficient soil moisture, may often markedly interfere with nodule formation and nitrogen fixation, but under reasonably normal soil conditions the carbohydrate supply seems to be the primary factor governing the intimate bacterial-host relations in legume symbiosis.

SUMMARY

This paper considers the rôle that carbohydrate plays in legume symbiosis from the time of bacterial entrance into the root until the nodule disintegrates, special emphasis being placed upon the response of the bacteria and host to varying supplies of carbohydrate. The more important facts gathered from many sources, which have a bearing on the subject, are brought together and correlated.

Nodule bacteria enter plant roots under almost any condition of carbohydrate concentration, but good nodule development is dependent upon a rather abundant carbohydrate supply in the roots.

The manner in which the carbohydrate is utilized, as the rate of photosynthesis varies, is considered in detail. If the carbohydrate is very limited, most of it is used for respiration, but as the supply increases growth becomes more abundant. At a carbohydrate level sufficiently high to permit considerable root growth, good nodule development normally takes place. If carbohydrates are supplied to the root, an abnormally large development of roots and nodules with respect to tops may occur.

The location of nodules is affected by the carbohydrate supply. Normally, nodules on annual legumes are located chiefly in the vicinity of the main roots and near the surface, assuming uniform inoculation and good soil conditions. On the other hand, they are usually widely scattered on plants grown either in water cultures, or in a nitrogen-free atmosphere, or in increased carbon dioxide, and when produced by ineffective strains. In all of these cases carbohydrate is abundant throughout the root system.

The physiological condition of the nodule, whether of the healthy, nitrogen fixing type, or whether at the stage where disintegration is starting, is also dependent in a large measure upon the carbohydrate supply. Other factors such as disease may, of course, produce nodule decay in the presence of abundant carbohydrate. The probable reasons why legume bacteria do not ordinarily attack nodule tissue well supplied with carbohydrate are considered.

The failure of nodules to develop abundantly in the presence of excess available nitrogen is due primarily to decreased carbohydrate supply in the roots. Under these conditions the carbohydrate-nitrogen ratio is narrow and an unusually large percentage of the carbohydrate synthesized is used for top growth, leaving less for the growth of roots and nodules. Good nodule development takes place only provided there is adequate carbohydrate for good root growth.

The quantity of nitrogen fixed by legumes growing under conditions favorable for fixation, usually, but not always, varies closely with the carbohydrate supply; the fixation roughly parallels the growth of the host tissue.

A consideration of the limited data, dealing with the energy requirements for the chemical process of nitrogen fixation as distinguished from the respiration and growth requirements of the bacteria and host, shows with a fair degree of certainty that they are negligible. The bulk of the carbohydrate appears to be consumed in respiration and growth, chiefly of the host.

These studies show very definitely that under reasonably normal conditions and with effective strains, the intimate relations existing between the nodule bacteria and their hosts in legume symbiosis are largely dependent upon the carbohydrate supply. If the supply is adequate, normal nodule activity usually follows; if the supply then becomes deficient, the bacteria sometimes merely remain more or less dormant in the nodule, or in other cases may attack the tissues of the host to obtain food. Under special conditions other factors, such as calcium deficiency, high acidity, and insufficient soil moisture, may, of course, outweigh carbohydrate supply in importance.

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A RESPONSE OF CHLOROTIC CORN PLANTS TO THE APPLICATION OF ZINC SULFATE TO THE SOIL[1]

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The physiological disease of corn locally called "white bud," a form of chlorosis, is widely distributed in central Florida. It is found chiefly in the fields which have been under cultivation for a number of years, but in some instances occurs on the poorer types of recently cleared land. It is widely distributed on the lighter sandy soils but also occurs on the heavier soils when they are subjected to excessive cultivation and continuous culture. The soils on which this injury has been observed to develop on corn include the Norfolk, Gainesville, Hernando, and Blanton series. In some fields the injury has been so acute that corn has failed completely unless large quantities of animal manures were used or the land was allowed to lie idle and grow up in weeds for a number of years. An extreme case of this trouble exists on the Florida Experiment Station farm at Gainesville. Here, on Norfolk medium fine sand and on Hernando medium fine sand, "white bud" has been exceptionally serious for the past several years. The land on this farm has been under continuous culture for years. Neither cover crops nor liberal applications of mixed commercial fertilizers have materially improved the chlorotic condition of the corn.

DESCRIPTION OF "WHITE BUD" OR CHLOROSIS OF CORN

Within a week after the emergence of the corn seedlings growing in affected soil, symptoms of chlorosis begin to show. The full development of chlorophyll in the older leaves scarcely takes place before light yellow streaks appear between the veins. Small white spots of inactive or dead tissue develop rapidly in the leaves, while small white areas which never develop chlorophyll are sometimes prevalent. The unfolding leaves in the buds of corn seedlings are often white to a very light yellow in color, giving rise to the use of the term "white bud" by farmers. These symptoms are shown in plate 1, figure 1.

On exceptionally bad areas, the corn seedlings seldom recover from the chlorosis. The older leaves die, and yellow to white leaves continue to unroll. Before the older leaves die they develop a number of dead areas ranging in color from light slate to dark brown. These areas often grow larger and merge until the whole leaf dies. Plate 1, figure 2 shows such a corn plant.

Other advanced stages of chlorosis or "white bud" are illustrated in plate 1, figures 3 and 4.   Figure 3 (pl. 1) shows an entire corn plant in which the lower older leaves are practically dead, and in some places the leaf tissue has actually fallen away or contracted so as to give the leaves a distorted appearance.   The upper leaves show typical yellow striping between the veins.   The internodes of the plant are definitely shortened, and the growth is stunted.   It is apparent that this diseased corn plant, which is already tasseling, will probably not produce a healthy ear of corn.   Figure 4 (pl. 1) illustrates some of the advanced stages of injury to the corn leaves as compared with the appearance of a healthy leaf.   These more advanced symptoms of the chlorosis are usually found in the lower leaves of the affected plants.   These leaves, in most cases, die long before the maturity of the plant.

Examination of the root systems of chlorotic and apparently healthy plants at different stages of growth have failed to reveal any outstanding differences. Both "white bud" and healthy plants evidently develop healthy root systems during the early stages of growth.

Field tests[3] with different varieties and selections of corn have shown that the varieties and selections developed on the poorer sandy soils are more resistant to "white bud" than those developed on the more fertile soils.   However, even the more resistant varieties become chlorotic on the worst affected areas.

The date of planting does not appear to have any definite effect on the ultimate development of chlorosis in the corn plant.   Corn planted in February and March grows slower than that planted in April, May, and June, and for this reason the symptoms in the earlier plantings are more readily observed and are apparently more pronounced.

Although an entire field of corn may show symptoms of the chlorosis in varying degrees, there are usually areas in which the plants are more severely affected than in others.   These areas are usually irregularly distributed over the field and are irregular in outline.   To date no correlation has been observed between the occurrence of these localized severely affected areas and any soil characteristic.

Various types of pot cultures of corn in the surface soils and subsoils from affected areas were made in the greenhouse and in the open.   City water was used on these plants.   In no instance did the "white bud" condition develop in the corn plants of these cultures.   Later, a quantity of the Gainesville city water was evaporated and the residue tested spectroscopically for the presence of some of the less abundant elements.   Zinc was found in relatively large quantities in the residue.   Traces of cadmium and copper were also detected. In view of the response of the "white bud" corn in the field to applications of zinc sulfate, as described in the following, it appears likely that the quantities

of zinc found in the city water were sufficient to prevent the development of "white bud." The field experiments were started in the spring of 1933.

METHOD OF FIELD EXPERIMENTATION

Tests were made on the Station Farm in a field that had produced chlorotic corn plants very uniformly over its entire area for a number of years. The soil is classified as a Norfolk medium fine sand with some small areas of Hernando medium fine sand. Within this affected area 3-foot by 6-foot plots were laid out in tiers with a 4-foot border around each plot. Each plot was enclosed to a depth of 8 inches with 1-inch by 10-inch cypress boards nailed together to form a frame. The frames were sunk into the ground by digging a trench around the block of soil in which the treatments were to be made and slipping the frame down around the block. This method proved quite satisfactory for the isolation of the plots but necessitated hand cultivation. The pH value of the surface soil and subsoil of the plots ranged from 5.58 to 6.35.

Four tiers of 40 plots each were established in this manner. All special treatments were made in quadruplicate, being replicated in each tier of plots. In each tier, 10 plots were maintained without special treatment making a total of 40 check plots. The plots were located so as to cover the variations in the soil as far as possible.

The general plan of the experiment included two sets of treatments involving (a) a mixed inorganic fertilizer made of nitrate of soda, superphosphate, and muriate of potash with and without mineral supplements and (b) a variety of organic materials in some instances in combination with mineral supplements. The different treatments and their rates per acre are given in table 1.

EXPERIMENTAL RESULTS

On March 22, 1933, the fertilizer treatments, organic materials, and supplements were applied in a row lengthwise of the small plots except where otherwise indicated. They were thoroughly incorporated with the soil in the row with a hoe. The 8–8–4 mixed inorganic fertilizer was made from nitrate of soda, superphosphate, and muriate of potash. It was applied uniformly to the plots receiving inorganic combinations at the rate of 400 pounds per acre. All materials were of the commercial grade used in fertilizers except the zinc sulfate, which was the chemically pure "analyzed salt" used in chemical laboratories. Only one-quarter of the nitrogen of the mixed inorganic fertilizer was applied at the time of the planting. The remainder was used as a side-dressing. On the same date nine Whatley Prolific corn seeds were planted in the row in each plot. Germination counts were made on April 6. Because of some injury to germination by certain treatments, the seedlings were removed from all plots on April 7, and the plots were replanted to the same variety of corn. Germination of this planting was satisfactory, and the young seedlings made a good start. Table 1 gives the treatments used and the germination percentages obtained from counts on the two plantings. In the first planting the

TABLE 1

Treatments applied to soil in studies of chlorosis or "white bud" of corn and their effect upon the germination of corn

TREAT-MENT NUMBER	MATERIALS APPLIED TO SOIL PER ACRE	PER CENT GERMI-TION	
		First test (a)	Second test (b)
1	Nothing* No special treatment	76.6	88.6
	Mixed inorganic fertilizer and supplements†		
2	8–8–4, Nitrate of soda, superphosphate, muriate of potash	61.1	94.4
3	8–8–0 + 200 pounds muriate of potash	44.4	94.4
4	8–8–0 + 375 pounds sulfate of potash-magnesia	36.1	88.9
5	8–8–4 + 400 pounds epsom salts	47.2	83.3
6	8–8–4 + 50 pounds manganous sulfate	66.6	94.4
7	8–8–4 + 200 pounds manganous sulfate	72.2	88.9
8	8–8–4 + 1,000 pounds dolomitic limestone	63.9	94.4
9	8–8–0 + 200 pounds muriate of potash and 200 pounds manganous sulfate	38.9	97.2
10	8–8–4 + 20 pounds zinc sulfate ($ZnSO_4 \cdot 7H_2O$)	77.8	80.6
11	8–8–4 + 500 pounds flowers of sulfur	83.3	91.7
12	8–8–4 + 500 pounds flowers of sulfur and 50 pounds manganous sulfate	63.9	94.4
13	8–8–4 + 1,000 pounds basic slag	83.3	86.1
14	8–8–0 + 375 pounds sulfate of potash-magnesia and 200 pounds manganous sulfate	47.2	86.1
15	0–8–4 + 50 pounds manganous sulfate and 178 pounds calcium nitrate	86.1	88.9
	Organic materials and supplements†		
16	2½ tons air-dried chlorotic *Crotalaria spectabilis* (broadcast)	72.2	94.4
17	2½ tons air-dried healthy *Crotalaria spectabilis* (broadcast)	72.2	94.4
18	2½ tons natural leaf mold from mixture of hardwoods and pine (broadcast)	77.8	83.3
19	2½ tons natural leaf mold (broadcast) and 50 pounds manganous sulfate (in row)	88.9	88.9
20	4 tons stable manure (horse)	63.9	88.9
21	2 tons chicken manure (without litter)	77.8	94.4
22	400 pounds Peruvian guano	69.4	88.9
23	5 tons acid peat from localized peat deposit near LaCrosse, Fla.	80.6	83.3
24	5 tons acid peat and 50 pounds of manganous sulfate	77.8	80.6
25	5 tons alkaline peat from Everglades Experiment Station, Belle Glade, Fla.	83.3	83.3
26	5 tons alkaline peat and 50 pounds manganous sulfate	72.2	91.7
27	5 tons alkaline peat and 20 pounds zinc sulfate ($ZnSO_4 \cdot 7H_2O$)	83.3	86.1
28	5 tons alkaline peat and 1,000 pounds basic slag	86.1	86.1

(a) Whatley's Prolific corn. Planted March 22, 1933. Germination count made April 6, 1933.

(b) Whatley's Prolific corn. Planted April 7, 1933. Germination count made April 19, 1933.

* Average of 40 plots. † Average of quadruplicated plots for each treatment.

germination of the corn seed was seriously injured by the larger applications of soluble magnesium and potassium salts and to a lesser extent by some of the other treatments. No injury to germination was observed in the second planting made approximately 2 weeks after the application of the materials.

TABLE 2

Percentage of corn plants showing "white bud" condition on plots receiving different treatments on dates indicated

TREATMENT NUMBER*	PER CENT CHLOROTIC PLANTS					
	April 19	April 24	April 28	May 2	May 6	May 8
1	85.8	97.8	94.9	94.2	100.0	100.0
2	67.6	100.0	58.9	41.2	94.0	100.0
3	86.7	100.0	55.9	61.9	100.0	100.0
4	87.4	100.0	62.5	68.8	96.9	100.0
5	86.7	96.5	55.2	41.4	96.6	100.0
6	91.2	100.0	47.0	23.5	100.0	97.0
7	96.8	100.0	53.1	43.8	84.4	100.0
8	85.2	96.9	63.3	50.0	100.0	100.0
9	91.4	100.0	63.7	45.4	90.9	100.0
10	86.2	100.0	81.5	33.3	22.2	3.7
11	69.7	84.9	48.5	27.3	81.3	96.8
12	85.2	94.4	54.3	54.3	100.0	100.0
13	90.4	100.0	29.0	41.9	100.0	100.0
14	93.6	100.0	28.1	12.5	37.5	68.7
15	90.7	96.9	40.7	43.7	96.8	100.0
16	85.2	100.0	66.7	90.9	100.0	100.0
17	67.6	100.0	100.0	85.3	100.0	100.0
18	83.3	100.0	74.2	58.0	76.7	56.7
19	71.9	96.9	21.9	41.9	54.5	64.5
20	71.9	9.4	0.0	21.9	25.0	0.0
21	64.7	64.7	35.3	67.6	66.7	84.8
22	90.7	100.0	96.8	100.0	100.0	100.0
23	86.7	100.0	86.2	86.2	100.0	100.0
24	82.7	96.5	53.6	35.7	96.3	92.9
25	96.6	100.0	43.3	40.0	87.0	80.0
26	87.9	96.9	35.5	35.5	90.3	83.9
27	93.6	100.0	40.2	12.9	51.7	3.3
28	77.4	90.0	6.7	46.7	100.0	100.0

* For materials applied to soil in each treatment, see table 1.

A number of observations were made on the occurrence of the chlorosis or "white bud" among the corn plants during the early stages of growth. The total number of plants and the number of chlorotic plants were counted. The results of these counts were calculated to percentages and are given in table 2. A large percentage of the very young corn seedlings were chlorotic, as seen from the count of April 19. Only the plants in the stable manure plots showed defi-

nite improvement by April 24. On April 28, the plants of the plots receiving an application of stable manure showed no chlorotic plants. A number of other treatments brought about a temporary improvement in plant condition during this period, but only the zinc sulfate supplement to alkaline peat and

TABLE 3

Average height of corn plants* in plots receiving different treatments on dates indicated

TREATMENT NUMBER†	HEIGHT IN FEET	
	June 2	June 29
1	0.8	3.1
2	0.7	2.8
3	0.9	3.4
4	1.2	4.3
5	1.1	3.9
6	1.0	3.7
7	1.0	3.5
8	0.8	3.0
9	1.1	4.2
10	1.7	5.9
11	0.8	1.9
12	0.9	3.3
13	0.6	2.6
14	1.7	5.6
15	1.1	4.0
16	0.9	3.4
17	0.9	3.9
18	1.4	5.7
19	1.7	5.9
20	2.8	7.0
21	2.3	6.6
22	0.9	3.6
23	0.9	3.1
24	1.1	4.0
25	1.4	4.7
26	1.5	4.9
27	1.9	6.4
28	1.1	4.2

* Whatley's Prolific corn, planted, April 7, 1933.

† For materials applied to soil in each treatment, see table 1.

the application of stable manure caused a plant improvement which was not transitory.

On May 2, the general condition of most of the plots receiving special treatment was relatively good. The plants of the plot receiving the zinc sulfate supplement to the mixed inorganic fertilizer application showed definite improvement for the first time on this date. On May 6, the plants showing the

least amount of the chlorosis were those in the plots receiving applications of the mixed inorganic fertilizer with 20 pounds per acre of zinc sulfate, the mixed inorganic fertilizer using sulfate of potash-magnesia and 200 pounds per acre of manganous sulfate, the stable manure plots and the alkaline peat plots plus 20 pounds of zinc sulfate per acre. The plants in plots treated with chicken manure and those treated with leaf mold showed a definite decrease in the amount of "white bud" during this period. On May 8, the plants treated with zinc sulfate and those treated with stable manure showed little or no chlorosis. The plants of the plots receiving an application of the mixed inorganic fertilizer with the potash from sulfate of potash-magnesia and 200 pounds per acre of manganous sulfate and those of the plots receiving applications of leaf mold showed definite improvement. The plants growing in plots treated with chicken manure also showed great improvement. They grew vigorously but during this period showed some evidence of "white bud."

After the count of the total number of the chlorotic plants on May 8, the stand was thinned to three plants per plot. A side-dressing of nitrate of soda was made to the plants receiving the mixed inorganic fertilizer on May 22. Height measurements of the individual corn plants in the plots were made on June 2 and June 29. The average heights of the corn plants in the differently fertilized and treated plots are given in table 3 for these dates.

From the height data of table 3, it may be seen that the application of 20 pounds per acre of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) with 400 pounds per acre of an 8–8–4 inorganic fertilizer made from nitrate of soda, superphosphate, and muriate of potash greatly stimulated the growth of the corn plants. The only other inorganic fertilizer combination which significantly stimulated the corn growth was the application of 375 pounds per acre of sulfate of potash-magnesia and 200 pounds per acre of manganous sulfate with the mixed inorganic fertilizer. Leaf mold, stable manure, and chicken manure produced vigorous plants as did 20 pounds of zinc sulfate in combination with alkaline peat at the rate of 5 tons per acre. Plate 2 illustrates the effect of the zinc sulfate in combination with a mixed inorganic fertilizer and with alkaline peat.

The corn plants were allowed to mature completely and the ears were husked from the stalks of each plot on September 11. The corn stalks were not harvested. The corn on the cob was thoroughly dried for 5 days at 100°C. The weights of the dried grain and cobs were obtained. The total number of stalks, the total number of ears, and the total dry weight of the corn produced on the quadruplicated plots of like treatment are given in table 4.

Among the completely inorganic combinations, the zinc sulfate supplement and the potash-magnesia-manganous sulfate combination with 400 pounds per acre of the inorganic 8–8–4 mixed fertilizer gave the largest and most significant grain yield. The zinc sulfate supplement gave much higher grain yields than the potash-magnesium sulfates with manganous sulfate. Stable manure, at the rate of 4 tons per acre applied in the row, increased the corn yield more than the other organic additions. Among the organic materials, alkaline

peat at the rate of 5 tons per acre with the zinc sulfate supplement was the most effective treatment in increasing the corn yield.   Two and one-half tons per acre of leaf mold with 50 pounds per acre of manganous sulfate, 2 tons per acre of chicken manure, $2\frac{1}{2}$ tons per acre of leaf mold, 5 tons of alkaline peat with

TABLE 4

The total yield of corn grown on quadruplicated plots -
(Dried at 100°C.)

TREATMENT NUMBER*	NUMBER		TOTAL WEIGHT		
	Stalks	Ears	Grain	Cobs	Grain & Cob
			gm.	*gm.*	*gm.*
1	11	6	204.6	57.9	262.5
2	8	2	51.0	17.0	68.0
3	11	3	59.0	28.0	87.0
4	11	12	462.0	128.0	590.0
5	11	10	345.0	93.0	438.0
6	12	9	409.0	108.0	517.0
7	12	8	167.0	51.0	218.0
8	10	3	105.0	28.0	133.0
9	11	9	298.0	78.0	376.0
10	12	18	983.0	252.0	1235.0
11	8	4	195.0	61.0	256.0
12	11	7	137.0	44.0	181.0
13	7	0	—	—	—
14	12	15	715.0	191.0	906.0
15	12	9	479.0	109.0	588.0
16	10	6	320.0	86.0	406.0
17	8	6	253.0	75.0	328.0
18	12	16	926.0	228.0	1154.0
19	12	18	1092.0	261.0	1353.0
20	12	22	1328.0	360.0	1688.0
21	12	22	955.0	397.0	1352.0
22	9	8	250.0	73.0	323.0
23	11	3	78.0	27.0	105.0
24	12	10	396.0	129.0	525.0
25	12	14	669.0	160.0	829.0
26	12	17	797.0	188.0	985.0
27	12	20	1261.0	277.0	1538.0
28	11	10	362.0	114.0	476.0

* For materials applied to soil in each treatment, see table 1.

50 pounds per acre of manganous sulfate were the other organic materials, (named in decreasing order), which materially and definitely increased the corn yields.   The application of 1,000 pounds of basic slag per acre in the planting furrow brought about an exaggeration of the "white bud" condition when used either with a mixed inorganic fertilizer or with alkaline peat.

REVIEW OF LITERATURE AND DISCUSSION

The importance of the less abundant elements in life processes has been recognized for a long time. With the full appreciation of the importance of these elements in biologic processes, the study of their distribution and function has been expanded until today an extensive literature has developed. For this reason, only a few of the publications treating of the zinc response of annual plants will be reviewed. Studies covering more specifically the zinc response of the corn and similar plants will be emphasized.

Brenchley (3) in 1927 gave an excellent review of the early reports on the effect of zinc on plant growth. Among these reports, the water culture studies of Mazé (8) and the field experiments of Javillier (5) on the effect of zinc compounds on the corn plant are of particular value for the present study.

Mazé (8) started the study of the effect of small amounts of several of the less abundant elements on the development of the corn plant in solution cultures in 1910, and continued them through several succeeding years. He used especially treated containers and purified salts. His early studies with zinc were evidently not very satisfactory but he reported that he obtained characteristic zinc-deficient plants in 1912. He described the leaves of the corn plants growing without a source of zinc as being darkened in color and having a metallic sheen. Nocturnal sweating became abundant, leaving deposits on the leaves and causing an incrustation. Soluble salts were found in the deposits, and the zinc-deficient plants were evidently not able to regulate the absorption of nutrients. The plants died from 3 to 5 days after the appearance of these symptoms. The corn plants grown in solutions without a source of zinc lived from 4 to 6 weeks after being placed in the solutions.

Among his numerous contributions to the zinc response in plants, Javillier (5), in 1912, reported field experiments in which crystallized zinc sulfate was applied simultaneously with appropriate fertilizers to wheat, corn, oats, lupine, and peas. One to ten kilograms (2.2 to 22 pounds of zinc sulfate per hectare (2½ acres) were used. Uniformly favorable results from the application of zinc sulfate to corn were obtained in the tests. The zinc sulfate increased the dry weight of the immature corn plants 18 to 25 per cent in some instances, while a 5 per cent increase was the smallest obtained. Results with other plants were irregular, though in some instances wheat and lupine responded favorably to applications of zinc sulfate.

From studies carried out in solution cultures under very carefully controlled conditions, Sommer and Lipman (10) added the sunflower and barley to the plants for whose healthy growth zinc is apparently essential. Sommer (9) added buckwheat, Winsor beans, and red kidney beans to the list of plants for whose healthy growth zinc is evidently essential in solution cultures. Miss Sommer points out that up to the present there are two plants of the Gramineae, two plants of the Leguminosae, and one each of the Compositae and Polygenaceae families which give evidence of the essential nature of zinc for their

healthy growth in solution cultures. McHargue and Shedd (7) studied the cumulative effect of compounds of manganese, copper, zinc, boron, and arsenic on the growth of the oat plant in sand cultures. They concluded that there was an increase in the yield of grain and straw when compounds of manganese, copper, and zinc were used as compared to the use of manganese compounds alone.

Allison, Bryan, and Hunter (2) reported transitory favorable response of a number of plants growing in the raw saw-grass peat of the Florida Everglades to the application of zinc sulfate. They suggested the possible use of zinc compounds in connection with compounds of the other less abundant elements. Acting upon this suggestion, Allison (1) reported a favorable response of the peanut plant in its first growth stages to applications of zinc sulfate to the raw Everglades saw-grass peat. However, the initial response in plant growth from zinc sulfate did not continue and the plants soon broke down. At the same time, it was possible to bring the peanut plants to an earlier maturity with the addition of zinc sulfate and copper sulfate together than with copper sulfate alone. The zinc sulfate and copper sulfate were used at the rate of 16 pounds and 50 pounds per acre, respectively, in the planting row.

The toxicity of zinc compounds in relatively low concentrations to higher plants has been abundantly established in simple solutions, nutrient solutions, pot cultures (especially with galvanized pots), and in soils contaminated with, or naturally containing, zinc compounds.

Some of the terms used to designate the favorable action of zinc compounds on plant growth are: the "catalytic" or "complementary" action, used by Javillier; the "essential" nature of zinc, implied by Mazé and Miss Sommer; the "stimulation of plant growth," preferred by Miss Brenchley; and the action of zinc in "precipitating soil toxins," suggested by Chandler, Hoagland, and Hibbard (4) in their study of the little leaf or rosette of deciduous and citrus trees in California. It must be admitted that even the very careful work of Mazé and Miss Sommer does not absolutely establish the essential nature of zinc for the healthy growth of all possible genetic variations of plants under all possible environmental conditions. Apparently, the nature of the favorable action of zinc on plant growth will remain unknown until more information has been accumulated.

Although the absolutely essential nature of zinc for the healthy growth of higher plants under all conditions may still be questioned, it must be admitted that many of the symptoms recorded by Mazé for his zinc-deficient corn plants have been further observed for the "white bud" corn plants under field conditions. Miss Sommer (9) reports that wheat and barley, members of the same plant family as corn, made a healthy growth in zinc-deficient nutrient solutions for about 2 weeks but subsequently stopped growing and gradually dried up. Field observations show that the response of corn seedlings suffering from "white bud" was delayed several weeks after the application of the zinc sulfate and the germination of the corn seeds.

It is apparent that the soil factors bringing about a condition of the corn plant called "white bud," a form of chlorosis, are not as yet known. However, the response of the young chlorotic corn plants to applications of zinc sulfate furnishes ample evidence that the presence of an available source of zinc or possibly closely associated elements will overcome the condition and lead to a healthy growth of the corn plant.

In the present studies[4] a qualitative spectroscopic test revealed the presence of minute quantities of magnesium, cadmium, lead, and copper in the "chemically pure" zinc sulfate. The manganous sulfate which gave a response when used in combination with a 375-pound per acre application of sulfate of potash-magnesia had detectable quantities of zinc when tested spectroscopically. On the other hand the double sulfate of potash-magnesia did not show the zinc line under the experimental conditions.

The acid peat, alkaline peat, stable manure, chicken manure, leaf mold, normal *Crotalaria spectabilis* and chlorotic *Crotalaria spectabilis* were ashed below 450°C., and the ashes were tested spectroscopically for the presence of zinc. All of these organic substances had detectable quantities of zinc in their ashes.

In the field tests with corn, stable manure, chicken manure, leaf mold, and alkaline peat brought about a definite favorable response of the chlorotic corn plants and an increase in yield. The acid peat and *Crotalaria spectabilis* were not effective. These differences in response suggest that there might be a difference in the availability of the zinc contained in the various organic materials. It is interesting to note in connection with the favorable response of chlorotic corn plants to applications of leaf mold that McHargue and Roy (6) report the presence of zinc in the leaves of 23 specimens of deciduous trees.

After the favorable response of the chlorotic corn to the applications of zinc sulfate to the soil was observed, leaves of affected corn plants were washed with a dilute zinc sulfate solution and injections into the stalks of badly affected plants were made. Neither of these treatments brought about a recovery of the affected plants.

<center>SUMMARY</center>

A chlorosis of the corn plant, locally called "white bud" is described.

Corn plants affected with "white bud" responded to a 20-pound per acre application of "chemically pure" zinc sulfate ($ZnSO_4 \cdot 7H_2O$) with a mixed inorganic fertilizer and with alkaline peat in the soil. The chlorotic plants regained their green color, made a healthy growth, and produced grain following the application of zinc sulfate.

Stable manure and leaf mold produced healthy corn plants in an affected

soil. Chicken manure and alkaline peat brought about a definite improvement in affected plants but did not cause a complete removal of "white bud" symptoms. Qualitative spectroscopic tests on the ashes of these organic materials showed the presence of zinc.

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PLATE 1

CHLOROSIS OF CORN

FIG. 1. Early symptoms of "white bud" in seedling of Whatley's Prolific Corn. Planted April 7, 1933, photographed April 24, 1933.

FIG. 2. Advanced stage of "white bud" in young Whatley's Prolific corn plant. Plants seldom recover from this stage. Planted, March 6, 1933, photographed April 24, 1933.

FIG. 3. Chlorotic Long Island Beauty corn plant showing striping of leaves. Planted, February 4, 1933, photographed, April 24, 1933.

FIG. 4. Leaves of "white bud" corn plant compared with healthy.

A. Reddish bronze color over entire leaf, green color practically dissipated.

B. Light slate-colored leaf practically dead.

C. Healthy leaf.

D. Green about midrib with light slate-colored edges.

E. Dead straw-colored leaf depleted of chlorophyll with exception of green streaked midrib.

Corn planted, March 3, 1933, photographed, April 24, 1933.

FIGS. 1-4

PLATE 2

EFFECT OF ZINC SULFATE IN COMBINATION WITH MIXED INORGANIC FERTILIZER AND WITH
ALKALINE PEAT ON DEVELOPMENT OF THE CORN PLANT ON SOIL AREAS WHICH PRODUCE
"WHITE BUD" OR CHLOROTIC PLANTS

Whatley's Prolific corn, planted April 7, 1933, photographed, June 22, 1933

FIG. 1. No treatment.

FIG. 2. Four hundred pounds per acre of a 8–8–4 mixed inorganic fertilizer made from nitrate of soda, superphosphate, and muriate of potash (in row).

FIG. 3. Five tons per acre of alkaline peat in the furrow.

FIG. 4. Four hundred pounds per acre of a 8–8–4 mixed inorganic fertilizer and 20 pounds per acre of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) (in row).

FIG. 5. Five tons per acre of alkaline peat in the furrow with 20 pounds per acre of zinc sulfate ($ZnSO_4 \cdot 7H_2O$).

FIGS. 1-5

THE LAWS OF SOIL COLLOIDAL BEHAVIOR: XVI. THE CATION EXCHANGE-MAXIMUM IN ALUMINO-SILICATES[1]

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The work on the isoelectrically precipitated alumino-silicates previously reported (1, V) showed that the cation exchange capacity increased with the proportion of silica in the complex. Aluminum hydroxide, isoelectric at pH 8.1, did not show any cation exchange at pH 7.0, and silica gel possesses this capacity to a very moderate extent. It was obvious, therefore, that the power to adsorb and exchange cations must show a maximum at a certain proportion of silica to alumina.

In the isoelectric precipitation, which was done in highly dilute systems (1, III), the alumina would not carry down over 3 mols of SiO_2 per mol Al_2O_3. In more concentrated solutions any amount of silica in excess of this ratio can be forced to precipitate with the alumina, but then the gel always remained electronegative down to the lowest pH employed. At low pH the alumina redissolves. It is apparently not possible to continue the isoelectric series beyond 3 mols SiO_2 per mol Al_2O_3. We have therefore continued the series by precipitation from more concentrated solutions and at a pH high enough to insure complete precipitation of the alumina. The procedure was as follows:

Constant volumes of a 0.1 M Na_2SiO_3 solution were rapidly mixed with various quantities of a 0.1 M $AlCl_3$ solution containing sufficient HCl to give a final pH between 5.0 and 5.5 as checked by methyl red. The mixture was filtered, and the precipitates were allowed to become air dry. They were then leached with neutral, N Ba-acetate until the filtrate was neutral, then treated with 10 cc. N $BaCl_2$ and washed until the Cl reaction disappeared. The Ba was then displaced by N NH_4Cl and the Ba determined in the usual way. The precipitates were then analyzed for SiO_2 and Al_2O_3. The adsorbed Ba could thus be calculated per millimol SiO_2. The results are given in table 137, which shows (*a*) the number of millimols of SiO_2 and Al_2O_3 in each precipitate; (*b*) the molar percentage (mols SiO_2 + mols Al_2O_3 = 100), (*c*) the molar ratio SiO_2/Al_2O_3, and (*d*) the number of milliequivalents adsorbed Ba in the total precipitate as well as per millimol SiO_2.

The exchange capacity previously (1, V) found for silica gel prepared from sodium silicate is included for comparison.   Figure 39 shows the relationship more clearly.

The results show a clear maximum in the cation exchange capacity at the ratio of 9 mols of SiO_2 to 1 mol Al_2O_3.   In a letter from A. J. Pugh who, together with M. S. duToit, has continued this work at the New Jersey Agricultural Experiment Station, we are informed that he has found the corresponding maximum in a ferric silicate series at a ratio $SiO_2/Fe_2O_3 = 4.2$.   The comparison is of interest, for it might help us to explain the cause of the phenomenom.

Mr. Pugh merely states that the maximum for the ferric silicate series was found at the molar ratio of 4.2.   The values for the exchange capacities of the

TABLE 137

The cation exchange capacity in alumino-silicates of various molar ratios

MILLIMOLS IN PRECIPITATE		IN 100 MOLS		$\dfrac{SiO_2}{Al_2O_3}$	Ba ADSORBED	
SiO_2	Al_2O_3	Mols SiO_2	Mols Al_2O_3		Total	Per m. mol SiO_2
					m.e.	*m.e.*
16.65	4.35	79.4	20.6	3.8	1.70	0.102
16.27	3.59	82.0	18.0	4.5	1.76	0.108
16.94	3.01	85.0	15.0	5.6	1.98	0.117
16.66	2.40	87.5	12.5	6.9	2.30	0.138
17.57	2.31	88.3	11.7	7.6	2.52	0.143
15.49	1.78	89.8	10.2	8.7	2.19	0.142
17.01	1.86	90.2	9.8	9.1	2.61	0.153
16.04	1.47	91.7	8.3	10.9	1.93	0.120
16.82	1.50	91.9	8.1	11.2	2.20	0.131
16.17	1.27	92.8	7.2	12.7	1.69	0.104
16.23	0.80	95.4	4.6	20.3	1.30	0.080
15.59	0.68	96.0	4.0	22.9	1.23	0.079
Silica gel..						0.016

series not being known to us, we are unable to plot the corresponding curve. But the actual position of the two legs of the curve as obtained by Pugh and duToit might be of doubtful value as far as a comparison with our curve representing the aluminum silicate series is concerned, because the properties of colloidal precipitates are often very much affected by the method of precipitation as well as by later treatment.   A comparative study can therefore be made only by a strict adherence to a uniform procedure.   If we assume, however, that the maximum exchange capacity in the ferric series is equal to that of the aluminum series then we have, together with the two points of origin, the three cardinal points on the ferric silicate curve.   On this basis we have plotted a curve (broken line) in our figure merely to indicate the position of the maximum in exchange capacity in the ferric silicate series.   (A ratio of 4.2 mols SiO_2 to

1.0 mol Fe_2O_3 corresponds on the basis of molar percentages to 80.8 mols SiO_2 and 19.2 mols Fe_2O_3.)

In the isoelectrically precipitated series it was found: (a) that the ferric silicates (as well as the phosphates and humates) possess a lower isoelectric point and a higher cation exchange capacity than the aluminum compounds of the same molar ratios; (b) that at the same isoelectric point the ferric complex contains less silica than the aluminum complex; (c) that ferric hydroxide has a lower isoelectric point than aluminum hydroxide; and (d) that ferric hydroxide when precipitated with bentonite reduced the cation exchange capacity of the latter to a much lesser degree than aluminum when so precipitated.

This could all be satisfactorily explained by the fact that $Fe(OH)_3$ is a weaker base than $Al(OH)_3$. The weaker base leaves a stronger acid residue, and this means a lower isoelectric point and a higher cation exchange capacity.
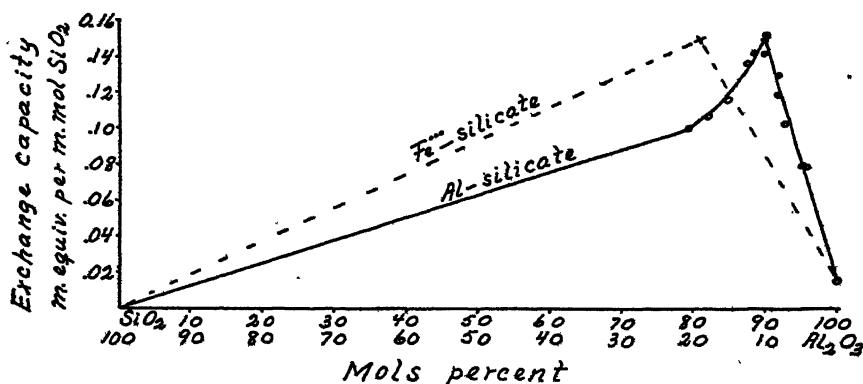
FIG. 39. THE CATION EXCHANGE—MAXIMUM IN PRECIPITATED ALUMINO- AND FERRIC-SILICATES

How are we now going to relate this difference in behavior to the two maxima in the cation exchange capacities?

The facts are: (a) that silica gel possesses a very moderate cation exchange capacity; (b) that in the presence of aluminum and ferric hydroxides (and also, as will be shown in a later publication, in the presence of magnesium) this capacity is substantially increased; and (c) that less of the stronger base $Al(OH)_3$ than of the weaker base $Fe(OH)_3$ is required to attain a maximum exchange (compare the right legs of the curves), but that beyond these points, that is, in higher proportions of the bases, the stronger base $Al(OH)_3$ suppresses the exchange power of the silicic acid more than the weaker $Fe(OH)_3$ (compare left legs of the curves).

It appears, therefore, that silicic acid is activated when in partial combination with a base, but that its activity or exchange capacity is again suppressed when the combination with the nondisplaceable cations of the base is more

complete. The fact that the isoelectrically precipitated ferric silicates (also phosphates and humates) possess a higher cation exchange capacity and a lower isoelectric point than the aluminum compounds of the corresponding molar ratios was explained on the theory that the acid residue is stronger in the ferric complex because $Fe(OH)_3$, being a weaker base, ties up a smaller number of acid valencies than $Al(OH)_3$. This we might indicate as follows:

$$\begin{array}{ccc}
\text{OH} & & \text{H} \\
\diagdown & & \diagup \\
& \text{Fe—SiO}_4\text{—H} & \\
\diagup & & \diagdown \\
\text{OH} & & \text{H}
\end{array} \qquad\qquad \begin{array}{c}
\text{H} \\
\diagup \\
\text{OH—Al}\!\!=\!\!\text{SiO}_4 \\
\diagdown \\
\text{H}
\end{array}$$

This relationship applies to the left legs of the curves in figure 39 where the proportion of sesquioxide is high. But it can equally well be applied to account for the right legs of the curves, for, if silicic acid is activated by a partial combination with the bases $Al(OH)_3$ and $Fe(OH)_3$, it seems obvious that the activation should proceed more rapidly with the stronger than with the weaker base since, in given quantities, the former would bind the greatest number of silicic acid molecules. Thus with a great excess of silicic acid, $Al(OH)_3$ might form:

$$\begin{array}{ccc}
& \text{SiO}_4\text{H}_3 & \\
& \diagup & \\
\text{OH—Al} & & \text{or} \\
& \diagdown & \\
& \text{SiO}_4\text{H}_3 &
\end{array} \qquad \begin{array}{c}
\text{SiO}_4\text{H}_3 \\
\diagup \\
\text{Al—SiO}_4\text{H}_3 \\
\diagdown \\
\text{SiO}_4\text{H}_3
\end{array}$$
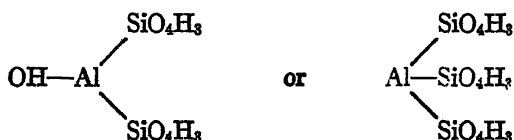
whereas $Fe(OH)_3$ would not combine to the same extent.

The fact that silicic acid yields a lower cation exchange capacity in the free condition than in the presence of certain bases might be accounted for if we assume that silicic acid is a pseudo acid which exists chiefly in its non-acidic form. In partial combination with a base with which a practically non-dissociated compound (an acid salt) is formed, a reversion into this non-acidic form would not take place. This would increase the *capacity* to combine with the displaceable cations. Its *strength* as an acid in the free condition appears, however, to be as great as, or greater than, in the partially combined condition because electrodialyzed silica gel showed a lower pH than any of a series of electrodialyzed alumino silicates (2, X).

Since the maximum in the cation exchange capacity must be assumed to coincide with a maximum in the acidic residue, it follows, by application of the aforementioned reasoning, that the maximum for the weaker base must lie at a lower molar ratio SiO_2/R_2O_3 than that of the stronger base. It requires, in other words, more of the weaker base to give rise to a maximum in acidic residue. With more base than corresponds to this maximum the acidic residue becomes neutralized and reduced (more by the stronger than by the weaker

base).   With more silica than that which corresponds to this maximum the acidic residue is reduced through a dilution by the less active free silica.

We can find no stoichiometric explanation for the fact that the maximum in the cation exchange capacity was found at a molar ratio of 4.2 in the ferric silicate series and at about 9.0 in the aluminum silicate series.   But should a stoichiometric relationship be expected in compounds so complex that each reaction unit contains thousands or millions of molecules and in which the surface composition may differ greatly from the composition of the total gel?

SUMMARY

The maximum in the cation exchange capacity of alumino-silicates has been found to be at a molar ratio of about 9 mols SiO_2 to 1 mol Al_2O_3.   A comparison is made with the corresponding ratio (4.2:1) of ferric silicates, and an explanation is offered on the basis of the general chemical and electrokinetic behavior of the two series.

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NOTE ON THE RELATION BETWEEN LIME CONTENT AND pH VALUES OF SOILS

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During the autumn of 1931 a large number of samples were taken for the purpose of making a soil survey, and determinations were made of lime content and pH, both in aqueous and in normal potassium chloride extracts of soils. The latter were determined colorimetrically according to the $BaSO_4$ method (5) using the complex indicators I and II as supplied by H. Jurany.[1]

The soils, which varied widely in their origin and composition, ranged from those with a high content of organic matter to those with none. Such widely different soil types as podzols and alkaline soils were also included so that as

TABLE 1

pH differences (H_2O and KCl) in extracts of soils in relation to the lime content

pH DIFFERENCE	NUMBER OF SAMPLES CONTAINING CALCIUM CARBONATE			TOTAL NUMBER OF SAMPLES
	0 per cent	0–0.6 per cent	>0.6 per cent	
<0.3	58	1	4	63
0.4–1.0	188	30	48	266
>1.0	22	9	34	65
Total........	268	40	86	394

wide a range as possible was obtained for the purpose of testing both the reliability of the pH methods as devised by the author and the variations in pH obtained by using potassium chloride for extraction.

As a result of applying the aforementioned methods of determining pH, it has been found that an interesting correlation exists between the divergent results obtained with the aqueous and potassium chloride extracts and the lime content of the soil. Because of the drift that occurs with the quinhydrone electrode, the regularity is not observed when the latter method is used (2, 3, 4).

The close relation between the lime content and pH difference may be seen from the data of table 1. The data prove that the great pH divergences oc-

[1] H. Jurany, Budapest IV, Váci utca 40.

167

cur with the alkaline, i.e. saturated, soils; minor divergences, with the limeless, i.e. unsaturated, soils.[2]

The surprising relation between the pH and pH difference may be seen in table 2 and figure 1. Neutral soils show a minimum divergence, and the

TABLE 2

pH of the soils

	NUMBER OF SAMPLES					
	7	31	100	82	34	27
pH..................................	5.0–5.4	5.5–5.9	6.0–6.4	6.5–6.9	7.0–7.4	7.5–7.9
Average pH difference with KCl and HP...........................	0.63	0.75	0.60	0.44	0.49	0.71

	NUMBER OF SAMPLES				
	39	34	11	26	3
pH..................................	8.0–8.4	8.5–8.9	9.0–9.4	9.5–9.9	10.0–10.4
Average pH difference with KCl and HP...........................	0.78	0.95	1.12	1.28	1.43
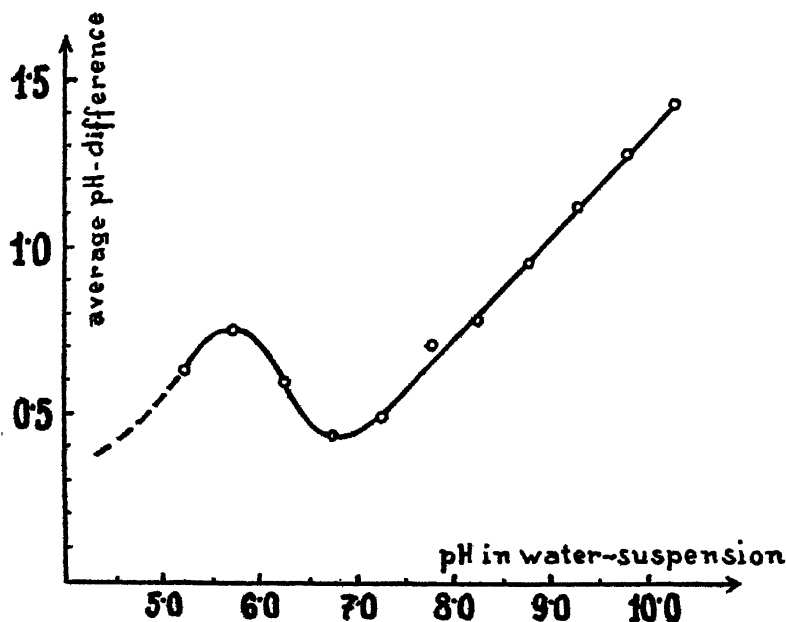
FIG. 1. RELATION BETWEEN pH VALUES AND AVERAGE pH DIFFERENCES

[2] There has been found a slight (<0.3 pH) difference with 268 limeless samples in 58 cases (21.6 per cent) and with 86 strongly calcareous soils only in 4 cases (4.7 per

difference is greatest with strongly alkaline soils. A point of inflection is obtained at pH 5.5 — 6.0 but the divergence diminishes at acidities greater than pH 5.5. Deines and Kleinschmit (1), however, found that at stronger acidities the average divergence is still less. Although no theoretical explanation can be given for the results, the regularity of the curve points to definite correlation between the aqueous pH and the pH as obtained in potassium chloride extracts. The data certainly refute the assumptions hitherto existing that small pH divergences occur with the saturated soils and great pH divergences with the unsaturated.

SUMMARY

A comprehensive examination of the pH results obtained in aqueous and 1N KCl extracts of 394 soil samples showed that:

Acid soils seldom show the large differences obtained with the highly alkaline soils.

A certain degree of regularity is observed between the average pH difference and the aqueous pH.

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cent). Great divergence (>1.0 pH) has been found in 22 cases out of 268 samples of limeless soils (8.2 per cent), and in 34 cases out of 86 samples of strongly calcareous soils (39.5 per cent); that is, five times as frequently.

A NOTE ON THE RELATIONSHIP BETWEEN THE CHEMICAL COMPOSITION OF SOIL COLLOIDS AND TWO OF THEIR PROPERTIES[1]

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Recent studies on the factors influencing the ratio of the moisture equivalents of soils to their permanent wilting percentages (4) have focused attention upon the chemical composition of the colloidal fractions of those soils.

Although Briggs and Shantz suggested a value of 1.84 for this supposed constant, more recent work by Veihmeyer and Hendrickson (9) demonstrates that no single value is acceptable for all soils. These workers report values ranging from 1.73 to 3.82. Local work (10) indicates that for Hawaiian soils a ratio of 1.30 is fairly accurate for most soils, although, as has been pointed out (19), each soil must be independently studied. It is significant that most of the soils reported by Briggs and Shantz[2] were obtained from the Great Plains Area; the highest ratio of Veihmeyer and Hendrickson (9) was from an Ohio soil; the low values obtained from local work are from soils which tend to be lateritic in view of the tropical conditions under which they were formed. Since the work of Gile (5), Anderson and Buyers (1), Robinson and Holmes (8), and others indicates that the chemical composition of the colloidal fraction varies with the conditions of weathering, a study of the physical significance of these variations was undertaken.

MOISTURE EQUIVALENT OF COLLOIDS AND THEIR CHEMICAL COMPOSITION

In 1930 Bouyoucos (2) suggested a direct proportionality between the colloidal contents of soils, as measured by the hydrometer, and their moisture equivalents. One is left with the impression that the water holding capacity of a soil, as measured by its moisture equivalent, depends upon the amount of colloidal material present, and that soil particles larger than the arbitrary limit for "colloidal material" act as simple adulterants and supply no water retaining power of their own. Apparently Briggs and McLane (3) had reached the same conclusion. On the other hand, Middleton (7) reported that although there was a general increase in the value of the moisture equivalent as the percentage of colloidal material increased, no precise relationship existed. It is significant that Middleton's results were based on 172 soils from various parts of the United States.

Bouyoucos' (2) results are plotted in figure 1. Here there is a significant straight line relationship for 11 of his soils. Moreover, the straight line when extended passes through the origin. But three of his points, which he dismisses as being "organic," are in themselves essentially collinear.
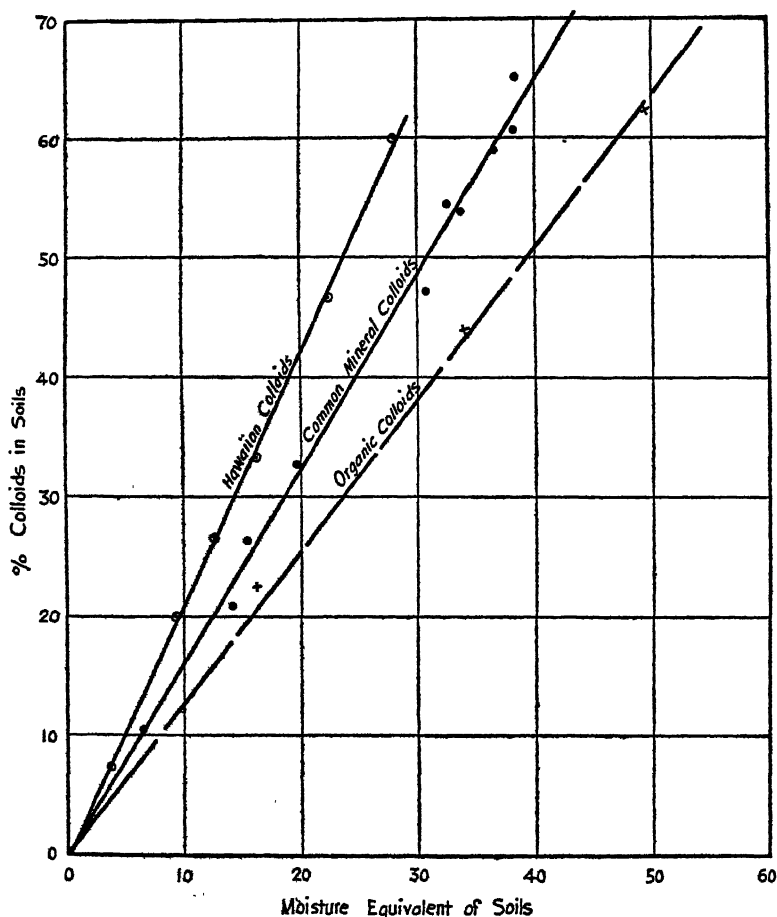
FIG. 1. RELATION BETWEEN THE MOISTURE EQUIVALENTS OF SOILS AND THE PERCENTAGES OF COLLOIDAL MATERIALS

Solid dots from general run of Bouyoucos' (2) results. Crosses from soils classified by Bouyoucos as "organic." Circles, "soils" formed by adding given amounts of extracted colloids from Hawaiian soil to silica sand.

Hawaiian soils do not respond readily to the hydrometer method because of the difficulty of obtaining comparable dispersion. Consequently "synthetic" Hawaiian soils were prepared by adding known percentages of extracted colloids to a base of inert silica sand of such grading that its effect

on the moisture equivalent might be ignored. Again the points are collinear, but although the line passes through the origin its slope is quite different from that of either of the other two.

If confidence can be placed in the few observations available for use in figure 1, one might conclude that the Bouyoucos proportionality between the colloidal contents and the moisture equivalents of soils would hold only if the colloidal materials involved in all cases were similar with respect to their water holding capacities. Furthermore, from the slopes of the three lines in figure 1 one might assume that unit mass of organic colloid held a relatively large amount of moisture at the moisture equivalent; unit mass of colloid from the Bouyoucos soils, less; and unit mass of colloid from Hawaiian soils, least. Since Hawaiian colloids are relatively lean in silica and rich in iron and alumina, it might be reasoned further that colloidal silica and organic matter had relatively high water retaining capacities but that colloidal iron and alumina did not.

Opportunity for testing this assumption is available in the recent work of Anderson and Buyers (1), who report the chemical nature of the colloidal material extracted from soils from six different series together with some of the physical properties of the same separates. The series are well selected, the Amarillo series being typical of the Great Plains, the Superior and Beckett series being classified as podzols, and the Davidson and Nipe series and bauxite representing semi-tropical conditions. In view of the samples obtained from various points in the profile, 24 samples are reported in detail.

Gile (5) and others have reported significant lineal correlations between the physical properties of soil colloids and the SiO_2/Al_2O_3 and SiO_2/Al_2O_3 + Fe_2O_3 ratios of those colloids. In the present case the correlation between the moisture equivalents of the 22 colloids available and the corresponding SiO_2/Al_2O_3 ratio is + 0.732 ± 0.070. Significant as this coefficient may be, it may be improved by the use of the more conventional molar ratio of silica to iron plus alumina. When these ratios are compared with the observed moisture equivalents, a correlation coefficient of + 0.751 ±0.062 is obtained.

This increase in the correlation coefficient naturally suggests that colloidal iron plays a part in fixing the moisture equivalent of the colloidal material. However, the use of Al_2O_3 + Fe_2O_3 in the denominator assumes that mole for mole iron and alumina carry the same moisture content when at the moisture equivalent. This may be far from the truth. Moreover, the organic matter, which seems to be high in water holding capacity, is still ignored.

A modification of the procedure permits the inclusion of all four variables which seem to dominate the water holding capacity at the moisture equivalent. In this procedure colloidal silica, iron, and alumina are assumed to exist as uncombined materials in the colloidal complex. Each is endowed with an unknown capacity for holding water at the moisture equivalent. The sum of each of these unknown capacities times the percentage of the proper

constituent of the colloid should give a measure of the moisture equivalents. Or

$$\text{M.E.} = x \text{ SiO}_2 + y \text{ Al}_2\text{O}_3 + z \text{ Fe}_2\text{O}_3 + w \text{ Organic}$$

where x, y, z, and w are the water holding capacities of SiO_2, Al_2O_3, Fe_2O_3, and organic matter respectively, and SiO_2, Al_2O_3, etc. represent the percentage of these materials in the colloid as disclosed by the analysis.

In the material at hand (1) we have the opportunity of forming 22 equations of this form. As has been indicated, the soils involved cover an extended range. The most plausible values for each of the unknowns may be determined by the method of normal equations. When this is done the following values are obtained:

Per cent

x = m.e. of colloidal SiO_2 in soil... 140
y = m.e. of colloidal Al_2O_3 in soil....................................... 31
z = m.e. of colloidal Fe_2O_3 in soil....................................... 61
w = m.e. of colloidal organic matter in soil............................... 116

If these results are acceptable it is evident that colloidal silica and organic matter are particularly important in determining the moisture equivalent of soil colloids, as has been pointed out in the discussion of Bouyoucos' results.

When these values are substituted in the original 22 analyses, we may compute the value of the moisture equivalent with a precision which, when compared with the observed values, measures the validity of the assumptions. When such computed values are compared with the observed values, a correlation coefficient of $+0.859 \pm 0.037$ is obtained. Such a high value, in view of the fact that the moisture equivalent of such finely divided material can be determined only with difficulty, may be considered as decidedly significant, particularly since all oxides of minor occurrence have been ignored.

EXCHANGEABLE BASE CAPACITY AND COLLOIDAL COMPOSITION

The data by Anderson and Buyers (1) permits the drawing of similar correlations between the exchangeable base capacity for the several colloids listed and their chemical composition in accordance with the method used in the foregoing. Figures for exchangeable base capacity are given in milliequivalents per gram of colloid by $BaCl_2$.

In comparing the usual $SiO_2/Al_2O_3 + Fe_2O_3$ ratio with exchange capacity in 23 cases, a correlation coefficient of $+0.855 \pm 0.038$ is obtained. If the simpler ratio of SiO_2/Al_2O_3 is used, the correlation coefficient increases to $+0.876 \pm 0.033$. Computing the ratio once more as $SiO_2 + Fe_2O_3/Al_2O_3$ reduces the correlation coefficient to $+0.574 \pm 0.094$. If significance can be claimed for these differences in the correlation coefficients, there seems to be some evidence that SiO_2 and Al_2O_3 differ widely in their base exchange characteristics, whereas Fe_2O_3 is relatively inert.

Resorting to the procedure used in the interpretation of the moisture equiva-

lent and assuming again that the materials exist in simple uncombined forms, we may write,

Base capacity $= x$ per cent $SiO_2 + y$ per cent $Al_2O_3 + z$ per cent $Fe_2O_3 + w$ per cent organic

Twenty-three cases reported provide an equal number of equations in this form. The procedure of normal equations again produces the most plausible value. When this is done

$$x = +0.01570 = \text{Exchangeable base per gram } SiO_2$$
$$y = -0.01044 = \text{Exchangeable base per gram } Al_2O_3$$
$$z = +0.00033 = \text{Exchangeable base per gram } Fe_2O_3$$
$$w = +0.00911 = \text{Exchangeable base per gram organic matter}$$

When these values are substituted in the 23 original equations and the computed base capacity is compared with the observed value, a correlation coefficient of $+0.919 \pm 0.025$ is obtained.

Significant as this relation seems to be, it is based upon values which are rather unexpected. The positive values for x, w, and z indicate that SiO_2, organic matter, and Fe_2O_3 possess base exchange capacities in the order named. The negative value for Al_2O_3 suggests that this material not only fails to possess base exchange capacity but actually tends to depress that characteristic in material adjacent to it. As an example, in the surface foot of the Nipe series a colloid carrying 10.2 per cent SiO_2 and 62.5 per cent Fe_2O_3 gave an observed base exchange capacity of only 0.031 m.e. Here the exchange capacity of 0.180, upon the basis of the values already given, is reduced to an observed value of 0.031, possibly because of the 15.8 per cent Al_2O_3 present.

If the negative sign for the Al_2O_3 may be accepted, it would be reasonable to suppose that this material is particularly or exclusively active in anion absorption. In fact, Matson (6) attacking the problem from an entirely different angle, reports that "with an increase in the silica content of the colloid we find an increase in the cation absorption and a decrease in anion absorption." And again, "As the SiO_2/R_2O_3 ratio increases, the colloids assume a more pronounced electronegative character. . . . . ."

SUMMARY

This paper is a statistical review of recent literature dealing with the relation between the chemical nature of soil colloids and their physical properties.

From the evidence available, there seems to be no reason for belief that SiO_2, Al_2O_3, and Fe_2O_3 exist in soil colloids in any form other than the simple oxides or hydrated oxides. Moreover, in so far as such statistical studies may be accepted, it would appear that each of these materials, as well as any organic matter which may be in colloidal form, possesses definite water holding characteristics at the moisture equivalent. When the chemical composition of a colloid is known, its moisture equivalent should be easily determined.

The base exchange capacity of colloids seems to be a function of their chemical composition as well. SiO_2, Fe_2O_3, and organic matter appear to absorb

these cations, whereas alumina seems to repel cations and possibly absorbs anions.

If it be true that the moisture equivalent of a soil is determined by the abundance and chemical nature of the colloidal material in it, it would seem reasonable to suppose that the moisture in a soil at the permanent wilting percentage is a function of the same variables. Consequently, a constant ratio between moisture equivalents and wilting percentages for all soils could exist only if the moisture held by each colloidal component were reduced by the same percentage during the growth of plants, prior to evidence of wilt.

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THE TOXIC INFLUENCE OF FLUORINE IN PHOSPHATIC FERTILIZERS ON THE GERMINATION OF CORN[1]

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Marked reduction in the percentage of germination of seeds of cultivated plants has often resulted from the application of fertilizers in contact with or near the seed. The causes and remedy for this injury have been the subject of numerous investigations. The Committee on Fertilizer Placement of the National Fertilizer Association (7) and coöperating agricultural experiment stations have accomplished much to remedy this objectionable effect by determining safe and effective methods of fertilizer application. Some excellent work has also been done to determine some of the causes of this fertilizer injury. It was believed, however, that there was need for further knowledge concerning this matter; therefore, the investigation described in this paper was undertaken.

HISTORICAL

The literature is replete with observations of retardation and inhibition of germination and growth caused by fertilizers and other chemical compounds. As early as 1733 Jethro Tull (14) noted that "too much nitre corrodes a plant." That was approximately one hundred years before the first shipment of Chilean nitrate from Iquique. Late in the nineteenth century an active interest was taken in the problem by French and German investigators. These early investigations were concerned chiefly with laboratory tests using solutions of single salts.

Claudel and Crochetelle (3) investigated the effect of KCl, K_2SO_4, $(NH_4)_2SO_4$, $NaNO_3$, ammoniated superphosphate, and basic slag on the germination of seeds. All of these materials, at the concentrations used in their experiment, except basic slag (which is relatively insoluble) retarded germination.

Vincent (15), working with wheat seeds and several soluble fertilizer salts, concluded that the ability to germinate was decreased with increase in concentration of the salt.

Much later, in 1921, Rudolfs (9) showed that the retarding action, with some exceptions, could be attributed to an interference with absorption of water by the seed as a result of the high osmotic concentration of the salt solutions.

Numerous other workers employing solutions and pot and field tests have supported the

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177

findings of these men.   Little doubt remains that high osmotic concentrations have a retarding effect on germination.

Injury to germination can be caused, however, not only by high osmotic concentration, but also by toxic substances.

Sigmund (12), working with peas, corn, and rape seeds, found that a 0.5 per cent solution of KF entirely prevented germination.

Bokorny (2) found that a 0.1 per cent solution of NaF was very injurious to cress seedlings and that a 0.1 per cent solution of HF completely prevented the germination of cress, barley, peas, flax, and bean seeds.

It is well known that phosphate rock contains a considerable quantity of fluorine.   The analyses by Jacobs and Reynolds (5) and also of Marshal and co-workers (6) show that the fluorine content of rock phosphate ranges between 3 and 4 per cent for most of the samples analyzed.   Moreover, the first mentioned investigators showed that most of this fluorine is carried over into the product during the manufacture of superphosphates, and the latter workers found that some fluorine is present in the phosphoric acid used for making treble superphosphate.

Allison (1), growing corn in tumblers, found that heavy applications of 18 per cent superphosphate prevented germination.

Rost (8), in pot and field work, found that both 16 and 46 per cent superphosphates were injurious to corn at heavy rates of application.   The 46 per cent superphosphate was more toxic in equivalent amounts than the 16 per cent superphosphate.   Contact with the soil for 1 month before planting largely overcame the toxicity of both fertilizers.

<div align="center">EXPERIMENTAL</div>

<div align="center">*Toxicity of superphosphates to germination of corn*</div>

To investigate the toxicity of various fertilizer materials, a method was used similar to that used by bacteriologists in testing the efficiency of disinfectants. The organisms (corn seeds in this case) were placed for a definite number of hours in the material investigated and then placed in a suitable medium for growth.   The effect of the treatment on viability was then compared with a control run parallel with the treated organisms.

Pastes were made by moistening fertilizers with distilled water.   Each paste was placed in a 2-pound glass butter dish having a loose glass cover.   One hundred seeds of corn were embedded in each of the pastes for the time designated.   Corn seeds of uniform size and high viability were used.   Any seeds that showed indication of mechanical injury or poor quality were discarded. After remaining in the paste for a definite number of hours, the seeds were removed, well washed with distilled water, weighed, and placed in "rag dolls" to germinate.   For the fluid treatments the same procedure was followed except that 500-cc. Ehrlenmeyer flasks, stoppered with absorbent cotton, were used as containers.

The "rag doll" consisted of a strip of cheesecloth 8 inches wide and 30 inches long.   The hundred seeds were spread uniformly on each strip and then rolled up with the cloth, a glass rod being used as an axis to give stiffness to the roll. Each "rag doll" was placed in a 5-pint jar containing 1 inch of distilled water so that the wick action of the cheesecloth moistened the seeds.   The jars were then connected by means of rubber tubing and aerated with moist air four times

daily. Germinated seeds were removed daily, and the number that germinated was recorded. As the reaction of the medium for plant growth may influence germination, the pH values of the pastes and solutions were measured by means of the quinhydrone electrode. The results of the experiment are given in table 1.

These results show that commercial ammonium sulfate and potassium chloride were injurious to germination. The toxic effect produced by the two superphosphates was especially severe. Immersion in these two materials for 3 hours was sufficient to reduce germination 75 per cent, and immersion for 12 hours killed practically all of the seeds. Similar toxicity was not shown by monocalcium phosphate, phosphoric acid, or sulfuric acid in the concentrations

TABLE 1

Per cent germination of corn after immersion in fertilizer materials

TREAT-MENT	SUBSTANCE	CONCENTRATION OF MEDIUM	pH OF MEDIUM	NUMBER OF HOURS IMMERSED					
				1	3	6	12	24	96
				Per Cent Germination					
1	H₂O (distilled)	....	....	..	..	..	..	98.5*	..
2	(NH₄)₂SO₄ (com'l)	Paste	4.23	..	..	..	93	85	88
3	KCl (com'l)	Paste	6.90	..	..	..	84	77	65
4	Superphosphate 20 per cent P₂O₅	Paste	2.48	87	27	7	1	0	0
5	Superphosphate B 44 per cent P₂O₅	Paste	2.41	86	24	9	0	0	0
6	Ca(H₂PO₄)₂ C. P.	Paste	1.56	..	..	..	..	91	..
7	H₃PO₄	0.1 M	1.61	..	..	..	..	99	..
8	Rock phosphate and H₂O	5 gm./100 cc.	7.16	..	..	..	..	97	..
9	H₂SO₄	0.143 N	1.20	..	..	..	..	95	..
10	Rock phosphate and 0.143 N H₂SO₄	5 gm./100 cc.	1.86	..	..	..	..	0	..

* Average of 10 determinations of 100 seeds each. Standard deviation = 1.12 per cent.

used. Neither was it shown by rock phosphate in water. When rock phosphate and 0.143 N sulfuric acid were mixed, however, a product was obtained that was highly toxic to germination.

Since retardation of germination due to high osmotic concentration of the medium is related to the water absorption of the seed, the weight of seeds before and after immersion in the fertilizer pastes was recorded. The per cent increase in weight of the seeds is shown in figure 1. It is at once apparent that the phosphates interfered with water absorption much less than did the more soluble ammonium sulfate and potassium chloride which, in turn, were less toxic than the phosphates. The interference with water absorption, then, does not afford an explanation of this injury.

The high acidity of the phosphates evidently did not account directly for their toxicity under the conditions of the experiment, because the more acid phosphoric and sulfuric acids did not produce a similar effect. Therefore, an explanation of the injury must be sought elsewhere.

Qualitative tests showed that concentrated sulfuric acid formed hydrofluoric acid from rock phosphate. When the mixture was heated a heavy
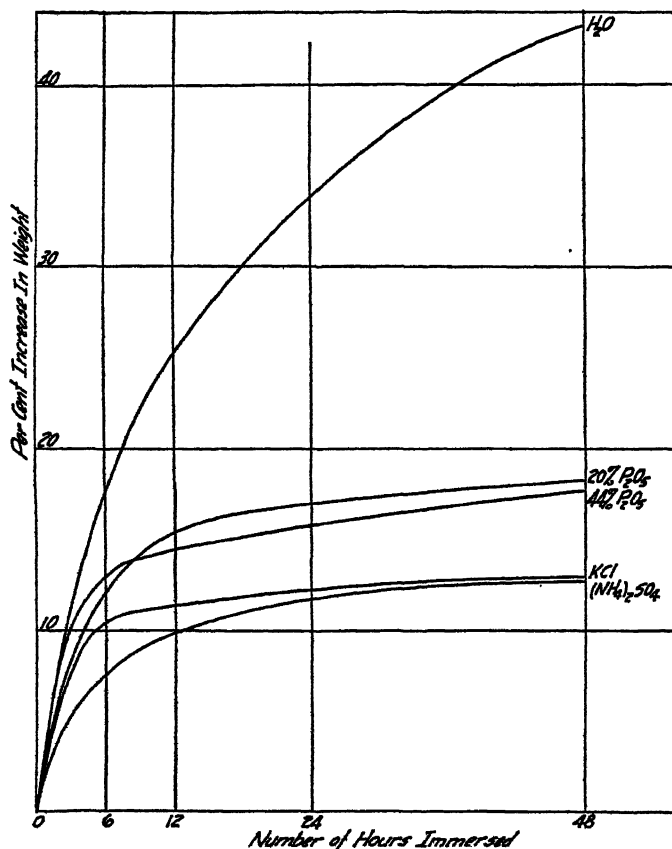
FIG. 1. PERCENTAGE INCREASE IN WEIGHT OF CORN SEEDS PLANTED IN MOIST FERTILIZER

etching was produced on a glass plate. Therefore, it seemed desirable to investigate fluorine as a possible source of injury to germination.

Toxicity of some fluorine compounds to germination of corn

Solutions of hydrogen fluoride and sodium fluoride were made containing known concentrations of fluorine. The strengths of these solutions were checked by titration with 0.1 N thorium nitrate using alizarin as an indicator, according to the method of Willard and Winters. The pH values of the solutions were determined by means of the quinhydrone electrode.

Four hundred cubic centimeters of each solution were placed in separate 5-pint jars. In each of these were placed two "rag dolls" each containing 100 seeds of corn. One of these "rag dolls" contained Polar Dent corn (the same variety used in the previous experiment), the other contained Pickett's Yellow Dent corn. In addition to the jars containing the fluoride solutions four more were added to the series. One of these contained 400 cc. of 0.143 N H_2SO_4; the second, an equal volume of 0.072 N H_2SO_4; the third, 400 cc. of 0.072 N H_2SO_4 plus 1 gm. of CaF_2 per 100 cc. of solution; and in the fourth jar solid CaF_2 was sprinkled directly on the seeds in the "rag doll" and 400 cc. of water was added. The jars were connected in series by tubing, aerated, and otherwise treated as in the first experiment.

TABLE 2

Influence of some fluorine compounds on the germination of corn

SUBSTANCE	FLUORINE	pH	PER CENT GERMINATION		TIME REQUIRED FOR GERMINATION
			Polar Dent	Pickett's Dent	
	p.p.m.				*days*
Check..............................	...	....	98.5*	99.2*	2.35
HF.............................	100	2.88	49	70	2.25
	200	2.66	9	22	3.00
	300	2.49	0	9	4.00
	400	2.40	0	0	
NaF.............................	100	5.47	61	72	2.00
	200	5.57	10	65	2.40
	300	5.61	12	23	2.95
	400	5.63	0	0	
0.143 N H_2SO_4........................	...	1.20	15	26	3.05
0.072 N H_2SO_4........................	...	1.43	68	67	2.10
0.072 N H_2SO_4 and 1 per cent CaF_2.....	...	1.44	0	0	
CaF_2 (solid)..........................	...		98	99	2.30

* Average of 10 determinations of 100 seeds each. Standard deviation 1.12 and 0.75 respectively.

It should be noted that this experiment and the subsequent ones differ from the first one in that the seeds were exposed to the reagents continuously through the wick action of the "rag doll," whereas in the first experiment the seeds were exposed to the reagents only for a limited time. The results are given in table 2.

Several salient facts were brought out by the data of the second experiment. Hydrogen fluoride and sodium fluoride were both toxic to germination of corn, and their toxicity increased with concentration. Hydrogen fluoride was more toxic than sodium fluoride. These results are in accord with the findings of Bokorny (2). He attributed the toxicity to the passage of fluorine into the seed where it united with the calcium present there to form insoluble calcium

fluoride. He states: "The calcium is an integral constituent of many cell organs and probably occurs as calcium proteinate in the same." His theory is further supported by his results on the effects of potassium oxalate and oxalic acid. Both these materials proved injurious in 0.1 per cent solutions, especially the latter, which killed the seeds of cress.

Sigmund, also, found that 0.5 and 0.3 per cent solutions of tartaric acid (another chemical which forms an insoluble compound with calcium) were injurious to wheat and killed pea and rape seeds. But Sigmund obtained no germination of peas, wheat, and rape when he used solid calcium or barium fluoride.

In the present investigation, however, repeated attempts to produce injury to corn by the use of solid calcium fluoride indicated that it had no effect on the germination of corn. Seeds of corn completely embedded in the moist salt germinated readily. When $0.072 N$ H_2SO_4 was added to calcium fluoride the results were entirely different. Then the material became highly toxic, allowing no germination to take place, probably as a result of the formation of hydrogen fluoride.

The strength of the sulfuric acid used also had an effect on germination. In the jar containing $0.143 N$ H_2SO_4, not only was the percentage of germination reduced, but the roots showed signs of severe injury, barely emerging from the seed, and the shoots were also injured but to a less extent. In some cases the shoot appeared before the root, which is the reverse of the natural process. The seedlings soon became covered with mold, and it was doubtful whether they would have survived if left to grow. The $0.072 N$ H_2SO_4 treatment proved to be injurious also, but the effect was relatively much less severe. Salter and McIlvaine have shown that a nutrient solution having a pH of 2.17 allowed 26 out of 30 corn seeds to germinate. The seedlings did not flourish, however, and eventually died. A solution of pH 2.96, on the other hand, allowed nearly perfect germination (29 out of 30), but growth was retarded.· Hence the acidity must be considered as a factor affecting germination.

It should also be noted that the corn seeds showed a marked variability with respect to their susceptibility to fluorine injury. For example, in the case of Pickett's Dent in the hydrogen fluoride solutions 9 per cent of the seeds were able to germinate in 300 p.p.m. of fluorine, whereas only 70 per cent of the seeds germinated in a solution containing 100 p.p.m. of fluorine. Moreover, the Polar Dent variety appeared to be more susceptible to fluorine injury than did Pickett's Dent in this experiment and also in subsequent ones.

The question arose as to whether the fluorine had a retarding effect on germination. The weighted mean (daily per cent germination times days) is very useful to express the time required for germination and is easily compared with the germination means from the other treatments. These values are found in the last column of table 2. The solutions of 100 p.p.m. of fluorine appear to have hastened germination to a slight extent, but above 200 p.p.m. a retarding action occurred. Similar effects can be observed in later experiments.

Relation of fluorine in superphosphate to germination

Because fluorine in fertilizers seemed to be a source of injury to germination and it is toxic apparently only in a soluble form, an investigation of the amount of soluble fluorine in fertilizers and its effect on germination was undertaken. Preliminary work showed that the quantity of soluble fluorine extracted from superphosphate was dependent on the quantity of water used and the length of time the fertilizer was shaken with the water. There was also a considerable difference in the amount of soluble fluorine in different superphosphates. Therefore, the following procedure was followed:

Two samples of 44 per cent superphosphate of different origin (which will hereafter be designated as A and B), and one sample of 20 per cent superphosphate were obtained from stock supplies used at this station. These samples were air dried in the laboratory and placed in suitable containers. Definite quantities of the air-dry fertilizer were weighed to 1 mgm. and added to 400 cc. of water in a 550-cc. glass bottle. These bottles were then stoppered and shaken for 6 hours in a shaking machine. Preliminary work had shown that 2 hours' shaking was sufficient to attain a maximum content of soluble fluorine in the extract, but 6 hours was allowed in order to insure complete equilibrium. After the shaking, the solutions were filtered and placed in stoppered bottles. Aliquots of the solutions were used to determine the freezing point depression by the usual Beckman thermometer method, the pH value by the quinhydrone electrode, and the soluble fluorine content by the method proposed by Willard and Winters. All these determinations were made in duplicate. Jars containing "rag dolls" were set up as before, and 200 cc. of solution was added to each. The germination test was run similarly to, and concurrently with, that of the pure fluorine compounds so that conditions of temperature, etc. were the same for both experiments. Table 3 gives the results of the tests.

Table 3 shows that superphosphate B was more toxic than the 20 per cent superphosphate, which in turn was more toxic than superphosphate A. The injury to germination produced by the solutions from the superphosphates increased with an increase in concentration of soluble fluorine. But, as might be expected, the acidity and osmotic concentration also increased with increasing amounts of fertilizer. In fact, the question might be raised whether or not the toxicity was entirely due to these last named factors. To answer this question one treatment was selected in which the values of these factors were the least. In the case of superphosphate B 1 gm. of fertilizer per 100 cc. of water gave a freezing point depression of only 0.19°C., which corresponds to an osmotic value of 2.28 atmospheres. Shive (11) obtained as good germination in solutions of 8.0 atmospheres as he did with the control cultures. The author found in further work with C. P. ammonium sulfate and potassium chloride solutions that corn seeds could germinate at much higher osmotic concentrations. Perfect germination was obtained in solutions of ammonium sulfate having a freezing point depression as high as 1.44°C. For potassium chloride the limit was still higher, 1.82°C.

The pH of the superphosphate solution under discussion happens to coincide with that of the solution used by Salter and McIlvaine on corn in which they obtained no reduction in germination. Also the slightly greater acidity of the 4-gm. solution of superphosphate A did not produce such toxicity. The fluorine content of the solution from superphosphate B was 154 p.p.m. The percentage of germination was approximately the same as that in 100 p.p.m. of fluorine as hydrogen fluoride (table 2). It must be true, therefore, that the toxicity of the fluorine in the superphosphate is modified by other factors.

TABLE 3

Per cent germination of corn in fertilizer solutions

FERTILIZER	GM. FERT. 100 cc. H₂O	FREEZING POINT LOWERING	pH	SOLUBLE FLUORINE	PER CENT GERMINATION		TIME REQUIRED FOR GERMINATION
					Polar Dent	Pickett's Dent	
		°C.		p. p. m.			days
Check......................	....	....	....	...	98.5	99.2	2.35
Superphosphate 20 per cent....	4	0.30	2.93	117	57	87	2.80
	8	0.53	2.75	172	35	41	3.60
	12	0.74	2.65	200	7	14	3.95
	16	0.91	2.59	243	1	3	5.00
	20	1.01	2.51	270	0	1	5.00
Superphosphate A 44 per cent..	4	0.52	2.94	147	91	96	2.30
	8	1.00	2.81	173	74	93	3.00
	12	1.34	2.76	214	64	68	3.85
	16	1.66	2.69	256	14	30	4.80
	20	1.98	2.66	327	3	3	4.85
Superphosphate B 44 per cent..	1	0.19	2.96	154	52	69	2.65
	2	0.30	2.91	263	22	33	3.80
	3	0.43	2.87	385	12	10	3.95
	4	0.53	2.84	513	1	5	4.80
	5	0.64	2.82	618	0	5	6.80
	6	0.77	2.77	670	0	0	

A comparison of the solution of superphosphate B having 2 gm. of fertilizer per 100 cc. with that of 20 per cent superphosphate having 4 gm. per 100 cc. of water shows that the osmotic values and acidity are practically the same, and that toxicity to germination varies directly with the fluorine content. Each of these solutions, however, was less toxic than corresponding solutions of hydrogen fluoride. In the three mentioned cases the pH of the solution was higher than that of the corresponding hydrogen fluoride solutions. The solution of 20 per cent superphosphate containing 12 gm. of fertilizer per 100 cc. contained 200 p.p.m. of fluorine, and its acidity is practically the same as the hydrogen fluoride solution of that strength. The injury to germination in

this case is slightly greater than that of the hydrogen fluoride solution. It appears, then, that the toxicity is favored by high acidity. This conclusion is further supported by the fact that hydrogen fluoride proved more toxic than sodium fluoride.

Had superphosphate A been omitted from the experiment the explanation of table 3 and some later experiments would have been greatly simplified, for its injury to germination is much less than would be expected from its soluble fluorine content. There were, however, three major differences between this fertilizer and the other two fertilizers; namely, the acidity was less and the soluble fluorine was less for a given weight of available P_2O_5 than in the case of the other two fertilizers; superphosphate A contained a small amount of copper as shown by qualitative analysis of the material, whereas no trace of copper was found in the other two fertilizers. There is need of further investigation to determine the exact cause for the lower toxicity of superphosphate A.

The time required for germination in the various solutions is given in the last column of table 3. Increasing amounts of fertilizer caused greater retardation of germination. The degree of retardation was not wholly controlled by osmotic concentration, but was affected by other factors as well. No doubt the fluorine content played some part in retarding germination.

Reduction of toxicity of superphosphates by the partial removal of fluorine

Although the results of the foregoing experiments indicated that soluble fluorine was responsible for the toxicity of the investigated superphosphates to the germination of corn, an exact interpretation of the results was complicated by the influence of other factors which may have had some effect on germination. An attempt was made, therefore, to remove the soluble fluorine from the superphosphate by a method which would cause the least possible alteration of the physical and chemical properties of the superphosphate used. For this purpose the volatilization method seemed best adapted because it did not require the addition of any foreign substance except water, and fortunately the fluorine distilled over at a low temperature (120°C.), which removed only a trace of phosphoric and sulfuric acid.

Twenty-four grams of superphosphate B was accurately weighed into a 400-cc. Ehrlenmeyer flask and placed in a constant temperature oven at 135°C. $\pm 2°$. The stoppered flask was connected to a water-cooled condenser on the outside of the oven by means of glass tubing. The superphosphate was alternately wetted and dried by the addition, through a capillary tube, of approximately 10-cc. portions of distilled water followed by several hours of drying at 135°C. The distillate was received in a suitable container. During 8 days of alternate wetting and drying 225 cc. of distillate was obtained. The hard cake of superphosphate formed by this procedure adhered to the flask so strongly that removal was not attempted, but the flask as well as its contents was broken up in a mortar, placed in a 550-cc. bottle, 400 cc. of distilled water added, shaken for 6 hours, and then filtered. The physical ap-

pearance of the fertilizer after being shaken did not seem to be different from that of the original superphosphate. The filtrate and the distillate from the superphosphate were analyzed and used for germination tests as in the preceding experiment.

Table 4 shows that not all of the soluble fluorine was removed from the superphosphate by this method, but it was greatly reduced in quantity. The reduction in soluble fluorine content was accompanied by a decrease in the toxicity of the superphosphate. The distillate from the superphosphate, on the other hand, was highly toxic to germination. This toxicity, no doubt, was due to the high concentration of fluorine which must have been in combination as hydro-fluosilicic acid because of the method used for separation. The osmotic concentration of the distillate was much too small appreciably to affect germination, and it has been shown in table 2 that 0.072 N H_2SO_4 having a pH of 1.43 was not as toxic as this distillate which had a pH of 1.89. Hence, the toxicity must have been due chiefly to the soluble fluorine present.

TABLE 4

Reduction of toxicity of superphosphates by the partial removal of fluorine

TREATMENT	GM. FERT. 100 CC. H_2O	FREEZING POINT DEPRESSION	pH	FLUORINE	PER CENT GERMINATION	
					Polar Dent	Pickett's Dent
		°C.		p.p.m.		
Check..................................	0	....	....	....	98.5	99.2
Superphosphate B (unheated).............	6	0.77	2.77	670	0	0
Superphosphate B (heated)...............	6	0.59	2.22	133	56	61
Distillate from heated fertilizer...........	..	0.05	1.89	1015	0	0

Effect of some soils on toxicity of fertilizer solutions to germination of corn

Results obtained with solution cultures are often quite different from those obtained if the solution is added to the soil. The soil usually reduces the toxicity of solutions and may completely overcome it. An experiment was run to determine whether this was true with fluorine in fertilizers. If the solution had been added to the soil the effect of the soil on soluble fluorine could not have been determined because of lack of suitable methods for extracting the soil solution. Therefore an intermediate method was chosen.

Solutions of superphosphate B were made in the same way as the solutions given in table 3 except that 50 gm. of soil (oven-dry basis) was added for each 100 cc. of water used. The procedure for shaking, analysis, and germination was the same as before. The germination tests ran concurrently with those of the aforementioned solutions. The results are given in table 5.

The addition of soil to the solutions caused a marked reduction in the soluble fluorine content and in the acidity. This effect was accompanied by a great change in the toxicity of the fertilizer. More than twelve times as much super-

phosphate B with soil added was required to produce the degree of toxicity obtained when 1 gm. of superphosphate B was used without soil (table 3). The reduction in fluorine content, no doubt, is due to the formation of insoluble calcium fluoride, which, according to table 2, is not toxic to germinating corn. Brookston loam was more effective than Miami silt loam in reducing the soluble fluoride content of the solution. The osmotic value of the solutions was not greatly changed. The retarding effect of the solutions is shown in the last column of table 5. Six grams of superphosphate B plus soil appeared to hasten germination, but the more concentrated solutions showed a retarding influence on the germination.

TABLE 5

Effect of soils on toxicity of fertilizer solutions to germination of corn

TREATMENT	GM. 44 PER CENT FERTILIZER 100 CC. H₂O	FREEZING POINT LOWERING	pH	FLUORINE	PER CENT GERMINATION		TIME REQUIRED FOR GERMINATION
					Polar Dent	Pickett's Dent	
		°C.		p.p.m.			days
Check..........................	..	....	....	...	98.5	99.2	2.35
44 per cent superphosphate (B)...	6	0.77	2.77	670	0	0	
	6	0.69	3.18	77	91	96	2.20
44 per cent superphosphate (B)	8	0.84	3.07	116	95	99	2.55
plus 50 gm. Miami silt loam	12	1.23	2.97	130	79	87	3.55
(pH 5.18) per 100 cc. solution.	16	1.52	2.88	177	38	35	4.70
	20	1.97	2.79	214	3	12	6.10
	6	0.68	3.96	12	95	96	2.05
44 per cent superphosphate (B)	8	0.85	3.69	22	96	95	2.35
plus 50 gm. Brookston loam	12	1.23	3.43	44	95	95	3.05
(pH 7.13) per 100 cc. solution.	16	1.50	3.19	70	60	62	4.30
	20	1.95	3.03	116	20	39	6.35

Relation of fertilizer placement to germination of corn in pot experiments

Since the effectiveness of soil in overcoming the toxicity of the superphosphates to corn had been determined, experiments were run in the greenhouse to investigate the toxicity of the fertilizers when placed in the soil with the seed. Two-gallon glazed jars were used, containing soil made up to definite moisture contents; 7,000 gm. of air-dry soil was used in the case of Brookston loam and 8,000 gm. in the case of Miami and Fox soils.

The rate of application of fertilizer was based upon a hill application in a circle 3 inches in diameter with the corn planted in check rows 3½ feet apart. By this method only 1/250 of the total area is covered. Hence, an application of 25 pounds per acre in the hill equals 25 times 250 or 6,250 pounds per acre

broadcast for the area covered. The fertilizer was applied with large salt shakers, the entire cross section of the soil being covered at the broadcast rates noted. Fifty seeds were planted per jar at a depth of 2½ inches. The jars were made up to weight every other day by adding water to the lower soil through a glass tube placed through the center of the soil column.

An experiment was conducted to determine what effect placing the embryo against the fertilizer would have upon germination as compared to the same treatment with the endosperm toward the fertilizer. Corn was planted on Miami silt loam at 10 per cent moisture content, 20 per cent superphosphate being used in contact with the seed. The placements and results are given in table 6.

Since the water was added to the lower part of the soil column the movement of moisture in the soil was predominantly upward as it would be in the field if no rains occurred during the time for germination. Under these conditions the fertilizer below the seed was injurious to germination of corn regardless of whether the fertilizer was in contact with the embryo or the endo-

TABLE 6

Relation of fertilizer placement to germination of corn in pot experiments

PLACEMENT OF FERTILIZER	PER CENT GERMINATION	
	12,500 lbs./A.	25,000 lbs./A.
No treatment..	98	100
Fertilizer below seed in contact with embryo.................	18	0
Fertilizer below seed in contact with endosperm..............	14	12
Fertilizer above seed in contact with embryo.................	14	10
Fertilizer above seed in contact with endosperm..............	82	42

sperm. The fertilizer above the seed produced greater injury when the embryo came in contact with the fertilizer than when the endosperm did. Plate 1 shows these jars 12 days after the seed was planted. Similar results with regard to facing the embryo of the corn kernel toward or away from the fertilizer were obtained by Coe, using Ammo-Phos in contact with the seed. The experiment indicates that when the diffusion of the fertilizer takes place in a direction away from the seed a few millimeters separation of seed and fertilizer may make a profound difference in the percentage of germination, for when the fertilizer was above the seed in contact with the endosperm, germination was much better than when the embryo was exposed to the fertilizer.

Influence of soil moisture on toxicity of fertilizers to germination of corn

Truog and his co-workers (13) found that the injury to germination of corn caused by a complete fertilizer varied inversely with the moisture content of the soil. An experiment was conducted to determine whether the same was

true for superphosphates.   Jars of Miami silt loam were made up to definite moisture content.   The fertilizer was applied beneath the seed in contact with the embryo.   Superphosphates A and B and 20 per cent superphosphate were included in this experiment.   The amount of fertilizer used in the case of the more concentrated carriers was such that an equivalent amount of available P_2O_5 was used in all three sets of pots.   Watering was accomplished in the same manner as before.   Daily counts were made of the number of plants emerging from the soil and from these the weighted mean for time of emergence was calculated.   Table 7 contains these results.

The data show that germination was both retarded and reduced by a decrease in soil moisture content.   Similar results were obtained by using Brookston loam at 20 per cent and 15 per cent soil moisture content.   The nature of

TABLE 7

Influence of soil moisture on toxicity of fertilizers to germination of corn

FERTILIZER	RATE OF AP-PLICATION	PER CENT GERMINA-TION		TIME REQUIRED FOR EMERGENCE	
		15 per cent soil moisture	10 per cent soil moisture	15 per cent soil moisture	10 per cent soil moisture
	lbs./A.			*[days*	*days*
Check....................................	......	98	100	5.0	5.6
Superphosphate 20 per cent...............	6,250	80	58	7.0	9.6
	12,500	50	14	8.6	10.0
	25,000	12	0	9.7	....
Superphosphate A 44 per cent..............	2,841	96	96	6.3	7.2
	5,682	84	80	8.2	8.7
	11,364	52	12	10.0	11.2
Superphosphate B 44 per cent..............	2,841	86	80	5.8	7.8
	5,682	44	48	8.4	8.8
	11,364	18	0	8.8	....

the experiment precluded reliable chemical analysis of the solution coming in contact with the seed, but from the laboratory results certain suppositions seem justified.   The lower moisture content of the soil probably resulted in a higher concentration of soluble material, including fluorine, at the surface of the seed.   Also, no doubt, there was a slower rate of reaction between fertilizer and soil at the lower moisture content than at the higher one, for diffusion in the soil was probably slower.   The combination of the two conditions named should have favored a higher soluble fluorine content as well as a higher osmotic concentration in the dryer soil, resulting in retardation and inhibition of growth.

Effect of mixing superphosphates with soils vs. direct contact with seed on germination

An experiment was set up to determine the effect on germination of mixing the superphosphates with the soil as compared to the placement of the same in direct contact with the seed. The jars for the direct contact treatments were made up as before, the fertilizer being placed below the seed in contact with the embryo. In the jars where mixing of fertilizer with the soil was desired, 1 kgm. of air-dry soil was mixed with the fertilizer, brought to the desired

TABLE 8

Effect of mixing fertilizer with soils on the toxicity of fertilizers to the germination of corn

FERTILIZER	RATE OF APPLICATION	PER CENT GERMINATION IN BROOKSTON LOAM		PER CENT GERMINATION IN MIAMI SILT LOAM		PER CENT GERMINATION IN FOX SANDY LOAM	
		Contact	Mixed	Contact	Mixed	Mixed limed	Mixed not limed
	lbs./A.						
Check............................	......	100	98	98	100	98	98
Superphosphate 20 per cent........	6,250	90	...	80	...	...	...
	12,500	46	98	50	98	100	100
	25,000	0	98	12	98	100	100
	50,000	0	100	0	100	100	100
	100,000	..	100	..	98	98	92
Superphosphate 44 per cent A......	2,841	98	...	96	...	...	...
	5,682	78	96	84	98	98	100
	11,364	16	100	52	100	100	98
	22,727	0	100	0	100	100	100
	45,454	..	100	..	94	8	6
Superphosphate 44 per cent B......	2,841	74	...	86	...	...	...
	5,682	56	100	44	98	100	100
	11,364	6	98	18	96	98	98
	22,727	0	98	0	98	100	98
	45,454	..	94	..	92	2	0
pH of soil..........................		7.13		5.18		6.48	5.10

moisture content, and placed in a layer over untreated moist soil. In this layer the corn kernels were planted. The layer of fertilized soil was approximately 1 inch thick and was covered by a layer of unfertilized soil. Three soils were used—Brookston loam at 20 per cent moisture content, Miami silt loam at 15 per cent moisture content, and Fox sandy loam at 12 per cent moisture content. The moisture content was approximately one-half of the moisture-holding capacity. The results of the experiment are recorded in table 8.

By mixing the fertilizer with the soil the toxicity of the fertilizer was almost

completely overcome except in the case of the heaviest application of the 44 per cent superphosphates on Fox sandy loam. More than sixteen times as much fertilizer could be applied without reduction in germination when the fertilizer was mixed with the soil as when it was used in direct contact with the seed. When the superphosphates were in direct contact with the seed the reaction between soil and fertilizer was not complete enough to overcome its toxicity.

It was supposed that the soil type might play an important part in the ability to overcome toxicity. Except for the heaviest application on Fox sandy loam, the results are rather unconvincing. The direct contact results indicate that reaction between soil and fertilizer was not sufficient to produce significant differences, whereas the reaction between the mixed soil and fertilizer was great enough to overcome toxicity in the majority of the cases.

The lower toxicity of superphosphate A is again brought out in the direct contact treatments of table 8. The difference between the toxicity of superphosphate B and 20 per cent superphosphate, however, was diminished as compared to the toxicity produced as shown in table 3. This can be accounted for by the fact that in the case of 20 per cent superphosphate, a greater weight of dry fertilizer was used which must have reduced the moisture content in the immediate vicinity of the seed.

Increased retardation of emergence of corn occurred with increasing amounts of fertilizer. The seed in soil mixed with fertilizer came up 1.3 days earlier as an average than when fertilizer was applied at the same rate in direct contact with the seed.

Toxicity of fertilizers to germination of corn in field experiments

The final experiment with respect to toxicity of superphosphates was conducted in the field. The fertilizer was applied in the hill in a circle 3 inches in diameter, and the seeds were planted in direct contact with the fertilizer. Amounts of the 44 per cent fertilizers equivalent in P_2O_5 content to the amounts applied in the 20 per cent superphosphate applications were used. For each treatment ten hills were planted with five kernels of corn per hill. At the time of planting, samples of soil were taken on which moisture content and pH were determined. Emergence counts were made at 12 and 21 days from date of planting. The final emergence is given in table 9.

The results of the greenhouse tests are confirmed by the field tests. There was a marked difference produced by facing the embryo toward or away from the fertilizer. The low moisture content probably accounts for the lower percentage of germination on Hillsdale sandy loam. Superphosphate A proved less toxic than superphosphate B or superphosphate 20 per cent. The percentage of germination, however, was much higher in the field experiment than in the greenhouse experiment. This may be due, in part, to the fact that only a circular area 3 inches in diameter was covered with fertilizer, whereas in the jars in the greenhouse the entire cross section of the jar was covered. The

plants that grew showed a marked tendency to send the roots through the soil beyond the fertilizer rather than through the fertilizer. Further comparison of percentage of germination between the greenhouse and field tests scarcely seems justified because different soils having different moisture contents were used and the climatic environment was entirely different.

TABLE 9

Toxicity of fertilizers to germination of corn in field experiments

OBSERVATION NUMBER	FERTILIZER	RATE OF APPLICATION	PER CENT GERMINATION		
			Fertilizer placed above seed vs. endosperm	Fertilizer placed below seed vs. embryo	Fertilizer placed below seed vs. embryo
		lbs./A.			
1	Check	....	98	92	94
2	Superphosphate 20 per cent	50	86	58	54
3		100	68	20	14
4		200	60	8	0
5	Superphosphate 44 per cent A	≈50	96	84	88
6		≈100	98	58	26
7		≈200	86	24	24
8	Superphosphate 44 per cent B	≈50	88	46	34
9		≈100	82	14	6
10		≈200	34	6	0
Soil type..			Hillsdale loam	Hillsdale loam	Hillsdale sandy loam
Soil moisture...............................*per cent*			20.3	21.3	10.4
pH...			7.86	7.81	7.84

SUMMARY

In this investigation, laboratory, greenhouse, and field experiments have shown that the superphosphates investigated were capable of exerting a toxic influence on the germination of corn as a result of their content of soluble fluorine. The soils used in the experiment were effective in reducing or overcoming the toxicity provided sufficient reaction took place between the soil and the fertilizer. These two facts furnish a satisfactory explanation of the high toxicity of superphosphates when placed in direct contact with the seed and also of the reduction of this toxicity when the superphosphates were mixed with the soil. Corn was found to be very variable in its susceptibility to fluorine injury. The facing of the embryo of the seed toward the superphosphate resulted in greater reduction in germination of corn than when the position of the kernel was reversed, provided the fertilizer diffused away from the seed. There was considerable difference in the amount of injury produced by the three superphosphates investigated. Further research is needed to determine the effects of nitrogen and potash carriers on the soluble fluorine content and on the toxicity of the superphosphates in mixtures of these fertilizers.

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PLATE 1

RELATION OF FERTILIZER PLACEMENT TO GERMINATION OF CORN

20 per cent superphosphate on Miami silt loam at 10 per cent moisture content. Fertilizer was placed above the seed.
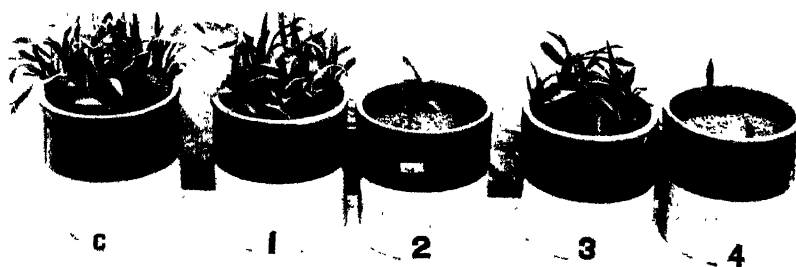
C—No fertilizer.

1—12,500 lbs./acre in contact with endosperm.

2—12,500 lbs./acre in contact with embryo.

3—25,000 lbs./acre in contact with endosperm.

4—25,000 lbs./acre in contact with embryo.

H. HOWE MORSE

ISOLATION OF SOME BACTERIA WHICH OXIDIZE THIOSULFATE[1]

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The sulfur bacteria have several physiological characteristics in common. They are autotrophic (5, 59, 66, 74) and make use of elemental sulfur or some inorganic incompletely oxidized sulfur compound as a source of energy. During their development, this specific sulfur substance becomes more completely oxidized, and the energy released by the reaction enables the bacteria to reduce dissolved carbon dioxide. The various organic constituents of the bacterial cells are constructed from these reduction products.

The members of this physiological group of bacteria are extremely diverse, particularly in morphological characteristics and to a surprising degree physiologically. In Bergey's Manual (9), the sulfur bacteria are classified under two such different orders as *Eubacteriales* and *Thiobacteriales*. According to van Niel (64), this latter order should be split into two orders, the *Rhodobacteria*—purple bacteria; and *Thiobacteria*—colorless sulfur bacteria. An additional group of pigmented forms is encountered in the green bacteria which appear to be most closely related to the purple forms in respect to their utilization of sulfide as a source of energy. The purple and green bacteria are distinctive physiologically from all other sulfur bacteria by their requirement for radiant energy in the form of light which is utilized through the agency of their pigments. The fact that they are anaerobic and that the purple bacteria are facultative autotrophs are further distinctive features (45, 64, 65). Present information suggests the likelihood that the physiological utilization of the specific inorganic sulfur substances is much the same by members of both the colorless *Thiobacteriales* and the *Thiobacillus* members of the *Eubacteriales* in spite of the pronounced differences in morphology of these two groups of organisms. Even the accumulation of sulfur within the cells of the larger *Thiobacteriales* has lost its early significance with the demonstration by van Niel (64) that, when sulfur is formed, it occurs within the cells of only the larger individuals and outside the small cells.

The sulfur bacteria were first characterized by Winogradsky (73) from examination of large forms, both pigmented and colorless. This characterization, having preceded the discovery of the group of small sulfur bacteria, probably led Omeliansky to distinguish as true sulfur bacteria only those larger forms that generally showed sulfur globules within the cells (47). In discussing the differences between Nathansohn's culture and the organisms obtained by Winogradsky, Omeliansky wrote, in 1904 (47, p. 239): "Ihre Oxydationskraft ist bedeutend schwächer als bei diesen, da sie nur imstande sind, Thiosulfate zu Tetrathionsäure und Schwefelsäure zu oxydieren. Auch Morphologisch unterschieden sie sich scharf von den echten Schwefelbakterien, da bei ihnen niemals intracelluläre Ausscheidung von Schwefel stattfindet. Man ist also wohl berechtigt, diese Gruppe von den echten Schwefelbakterien abzuscheiden und mit einem besonderen Namen zu belegen. Wir schlagen dafür 'Thionsäurebakterien' vor."[2] During the years which have since ensued it has been shown that some members of the genus *Thiobacillus* are able to oxidize sulfur and other sulfur compounds fully as energetically as is reported for at least some of the larger sulfur bacteria. As an

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example we might cite a case of oxidation by *Th. thiooxidans*. When large amounts of elementary sulfur were initially present in the culture solution (25 gm. sulfur to 100 cc. of solution) about 500 mgm. of sulfur were oxidized by this bacterium in 10 days (70). Typical purple and green sulfur bacteria studied by van Niel (64) oxidized from about 5 to 25 mgm. of H_2S in 2 weeks. However, it seems likely that oxidation of sulfide by species of *Thiobacillus* is less energetic and less representative of the group than with the larger sulfur bacteria. Intensity of oxidizing capacity does not seem to be a suitable criterion for separating the two groups of organisms. Even the presence of sulfur within the cells has lost its earlier significance.

A very limited conception of the sulfur bacteria is expressed in the recent book by Ellis. He states (14, p. 3), "The term sulphur bacteria embraces only those organisms which oxidize the H_2S to S, store the latter temporarily in their bodies, and then oxidize it to SO_4." Such a viewpoint is not only incompatible with our knowledge of the physiology of the *Thiobacillus* species, but it also fails to typify the higher sulfur bacteria.

There seems to be no valid reason why all of the bacteria which can utilize inorganic sulfur materials as the specific energy sources while developing as autotrophs, should not be classified as true sulfur bacteria. Morphological features certainly should not be the major criteria for determining whether or not a bacterium belongs to a specific physiological group.

Ellis also misappropriated the genus *Thiobacillus* for some members of the larger sulfur bacteria. He states (14, p. 130), "The former word (*Thiobacillus*), however, is frequently used loosely to indicate certain thionic acid bacteria, and in particular *Thiobacillus thiooxidans* which in the strict sense is not one of the sulphur bacteria. Up to the present, the term *Thiobacillus* has been used in the publications of American writers as a convenient catalogue name, devoid of any phyletic significance." In such statements he shows an extreme lack of appreciation of the activities of the *Thiobacillus* species. Far from being used loosely in referring to certain bacteria, the term *Thiobacillus* has been applied to a very specific group of bacteria since its introduction in 1904 by Beijerinck (7, 8), and this group includes only autotrophic sulfur bacteria of the order Eubacteriales. To state that *Th. thiooxidans* is not a sulfur bacterium clearly is an admission of misconception. This organism is a very typical bacterium which is strictly autotrophic and is able to obtain energy for its development only from elemental sulfur, thiosulfate, or similar incompletely oxidized inorganic sulfur compounds.

Since Omeliansky first applied the term "Thionsäurebakterien" to the group of bacteria now known as members of the genus *Thiobacillus*, it has been customary to consider them under this title. This implies certain ideas which are incompatible with the facts. Although bacteria of this group all appear to be able to oxidize thiosulfate, their activity is not confined to this substance and some of them also oxidize elemental sulfur and certain polythionates. On the other hand, the purple sulfur bacteria also oxidize thiosulfate in addition to sulfur, sulfide, and sulfite (64). Furthermore, thiosulfate appears to be fully as satisfactory as sulfide for the purple bacteria, since it can be used in higher concentrations than sulfide without injury to the bacteria. In view of these facts the terms "Thiosulfate Bacteria" and "Thionsäurebakterien" are incongruous in their present application and it would seem wise to discontinue their use.

It is with some members of the genus *Thiobacillus* that the studies of this and some following reports are primarily concerned. More specifically, the investigations deal with the characterization of some of these forms from the nature of their growth upon media containing thiosulfate. Hasty judgment might lead one to conclude that the examination of soils for organisms capable of oxidizing thiosulfate would lead to results which have little or no application in natural phenomena. Although thiosulfate is seldom applied directly to soils it may reach the soil from various sources. It appears in some spring waters as a product of the incomplete oxidation of sulfide (12). It may arise in a similar manner in natural black mud (5). Of greater significance is the probability of its formation as an intermediate

product during the oxidation of elemental sulfur in soils. The results of Guittonneau and Keilling clearly show that numerous heterotrophic organisms are able to oxidize sulfur to thiosulfate both in soils and in solution media (19–28). Further oxidation appears to be effected by the sulfur bacteria. Elemental sulfur is applied to soils for correcting soil reaction, and larger quantities reach the soil as residues from dusting and spraying. Guittonneau and Keilling calculate that at least 100,000 tons of sulfur are used each year to treat the French vineyards as a fungicide. In this country increasing amounts of sulfur are being used in the control of parasitic fungi and insects. As much as 320 pounds an acre are applied in a single season in some commercial apple orchards (42). Some thiosulfate may be produced during the transformation of sulfur which is contained in the plant and animal residues that reach the soil.

The importance of bacteria capable of oxidizing thiosulfate assumes broader significance when it is realized that many, or possibly most, of them are also able to oxidize other inorganic sulfur substances which occur in natural environments in greater quantities than thiosulfate. Furthermore, it is particularly desirable to isolate the organisms in thiosulfate media, since in many instances there is better development than upon other sulfur compounds.

In spite of the fact that a comparatively large number of reports have dealt with members of the genus *Thiobacillus*, in few instances has sufficient information been presented concerning the morphology and physiology of the bacteria to enable one to determine whether or not new species were being considered. Although there are but four species of *Thiobacillus* listed in Bergey's Manual, no less than 12 species names have been published, and an equal additional number of species could be distinguished in the list of organisms already described in the literature, if minor differences justified the creation of new species.

DISTRIBUTION

The first detailed study of these bacteria is generally credited to Nathansohn (46). By his culture obtained from sea water at Naples, the thiosulfate was decomposed to sulfate and elemental sulfur. The following reaction was proposed to explain the reaction:

$$2 Na_2S_2O_3 + O_2 = 2 Na_2SO_4 + 2 S \qquad (A)$$
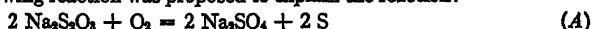
Since incompletely oxidized sulfur compounds appeared in the medium he finally concluded that the important reaction was:

$$6 Na_2S_2O_3 + 5 O_2 = 2 Na_2S_4O_6 + 4 Na_2SO_4 \qquad (B)$$
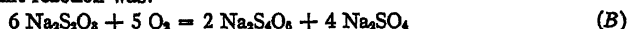
The precipitated sulfur was believed to arise from secondary reactions. If his conclusions were correct, he dealt with a transformation that has not since been described. It is conceivable that his culture was impure.

Beijerinck studied similar organisms obtained from fresh water, canal mud, and salt water (7, 8). Reaction (A) was believed to best represent the transformation. The name *Thiobacillus thioparus* was applied to the bacterium. Jacobsen isolated the same organism from soil, ditch water, sewage, and sea water and studied its oxidation of sulfur (33) and sulfide (34).

Organisms similar to *Th. thioparus* were obtained by Issatschenko, from the sea mud of the Arctic Ocean (29); by Brenner from marine mud of Kiel, rich in H_2S (10); and by Guittonneau from soil in France (20, 21, 22, 24).

Cultures which may have been identical with *Th. thioparus* were obtained by Waksman et al. from an alkali soil from California (71); by Klein and Limberger from ditch mud, composts, beach sand, and various soils of Germany (38); by Klein and Steiner in the mud and water of Lake Lunz in Germany (39); by Szimmat in garden soil, alder bog, and moor soil of East Prussia (60); and by Wudtke in 29 of 85 soils of waste land near the Baltic Sea (75). Organisms effecting the oxidation of thiosulfate in much the same manner as *Th. thioparus* were detected by Joffe in nine New Jersey soils and in eight from Oregon. The only soils not indicating presence of the organism were soils which had become very acid subsequent to the addition of sulfur (36).

These facts seem to indicate that *Th. thioparus*, or species closely related to it, are widely distributed in nature.

Beijerinck studied a second organism obtained from canal water, mud, and salt water. It developed in the absence of free oxygen and oxidized thiosulfate or sulfur, causing the reduction of nitrate to gaseous nitrogen (7, 8).

$$6 \ KNO_3 + 5 \ S + 2 \ CaCO_3 = 3 \ K_2SO_4 + 2 \ CaSO_4 + 2 \ CO_2 + 3 \ N_2 \qquad (C)$$
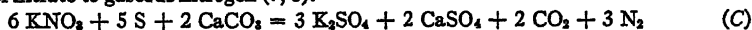
This organism was named *Th. denitrificans*. It was believed to be a facultative autotroph, but from the results of Lieske (41) and Gehring (17), it seems likely that it is strictly autotrophic. Lieske obtained his cultures from mud of the Leipzig Botanical Gardens. Gehring, working at Göttingen, found the organism very generally distributed and detected it in cultivated soils, earth composts, beech forest soils, peats, and mud. More recently, Tjulpanova-Mossevitch recovered similar bacteria from two fresh water and four salt water basins in Russia (61). These cultures were facultative anaerobes and facultative autotrophs! *Th. denitrificans* appears to be widely distributed but, because of difficulties of isolation, has been less generally recognized than *Th. thioparus*.

A third species was isolated from sulfur-soil-phosphate composts by Waksman and Joffe (67, 68). This organism, named *Th. thiooxidans*, oxidizes thiosulfate and sulfur to sulfate and develops under extremely acid conditions.[2]

$$2 \ S + 3 \ O_2 + 2 \ H_2O = 2 \ H_2SO_4 \qquad (D)$$

Brown obtained a similar organism from garden soil, activated sludge, and soil-sulfur compost (11). Joffe recovered the organism only from soils which had been treated with sulfur (36). The bacterium has more recently been found by Jensen in a dried up sea bed in Denmark (35), by Drewes in a bog about Kiel (13), and by Waksman in waters of a Mexican sulfur spring (6). *Th. thiooxidans* was detected in only 1 of 55 peat soils of Minnesota studied by Skinner and Nygard, and this soil had previously been fertilized with flowers of sulfur (56). In a garden soil, a moor, and an alder bog, Szimmat found organisms which were believed to be *Th. thiooxidans* (60). Wüdtke claims to have encountered the bacterium in 22 of 85 soils in East Prussia (75). Wilson and Higbee reported that organisms of similar character occur in most of the important mineral soils of New York State (72). In these last three reports the results are too meagre to indicate whether or not *Th. thiooxidans* occurred in the soil populations.

Ayyar, Perumal, and Norris working in India obtained a culture from activated sludge which reacted much the same as *Th. thiooxidans* (2, 3, 4). One of the cultures isolated by Aquino from a soil in the Philippines was believed to be *Th. thiooxidans* (1). Since the organisms of both Aquino and Ayyar developed upon organic media, they are not identical with *Th. thiooxidans*. It is possible that they were using mixed cultures.

Rountree isolated from five different soils of South Australia an organism which reacted much the same as *Th. thiooxidans* (49). It differed from *Th. thiooxidans* in size and in the range of hydrogen-ion concentrations over which it developed. Emoto described and named four species which were obtained from thermal sulfur springs in Japan (15, 16). These were called *Th. thermitanus*, *Th. lobatus*, *Th. crenatus*, *Th. umbonatus*. The only features which distinguished one of these species from another or from *Th. thiooxidans* were differences in size of cells and minor variations in appearance of colonies on solid media.

Trautwein obtained cultures from sewage purification plants, the River Tauber, and soils. They were described as facultative autotrophs which formed sulfate and polythionates from thiosulfate (62, 63).

$$4 \ Na_2S_2O_3 + 6 \ O_3 = 4 \ Na_2S_2O_6 \qquad (E)$$
$$3 \ Na_2S_2O_3 + 5 \ O = Na_2S_4O_6 + 2 \ Na_2SO_4 \qquad (F)$$
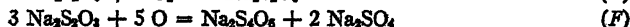
These bacteria were distinctly different from any of the forms previously described as sulfur bacteria. They more closely resembled *Bacterium denitrificans* Stutzer and Burri (L & N).

Halophilic cultures closely resembling *Th. thioparus* have been obtained from various salt seas and lakes in Russia and Siberia. Issatschenko and Salimowskaja describe two species and one variety: *Th. Beijerinckii*, *Th. Nathansonii*, and *Th. Beijerinckii* var. *Jacobsenii* (31,

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[2] For complete list of studies of this organism see references 57, 58.

32). They differ somewhat in cell size and tolerance to salt concentrations. Such organisms were detected in all 10 of the salt seas studied, in the Arctic Ocean, Tambukan sulfate lake, and soda lakes of Siberia (30, 40).

Other halophilic forms similar to those obtained by Issatschenko were isolated by Saslawsky and associates (51-55). One form came from the Kujalnitzky Liman and a second from the Ssuchoi Liman, the second oxidizing the thiosulfate completely to sulfate and the first leaving some unoxidized material.

Several additional cultures have been obtained which do not seem to be identical with any of those mentioned in the foregoing. Salimowskaja's organism was isolated from the river Fontanka (50). It developed much the same as *Th. thioparus* except that it was favored by a moderate acid reaction and brought about an increase in pH of the cultural medium during growth. Lockett studied the oxidation of thiosulfate by microörganisms in systems much like bacterial sewage filters (43, 44). Some bacteria recovered from the filters decomposed thiosulfate to sulfate in media containing gelatin or Rochelle salt, but failed to grow on the common laboratory media in the absence of thiosulfate.

A very tiny coccoid bacterium which was able to break down thiosulfate to sulfur and sulfate was recovered by Paine, Linggood, Schlimmer, and Thrupp from corroding walls and building stones (48). This organism was believed to play an important rôle in the disintegration of the masonry.

Gicklhorn named and described two organisms (*Bacterium cristalliferum* and *Bacterium retiformans*) which were developing in sulfide water (18). They were not obtained in pure culture, and no determination was made of their action upon sulfur compounds, but it was believed that they were sulfur bacteria.

Included in the assortment of transformations which have been ascribed to sulfur bacteria is the following, proposed by Beijerinck in 1904 but receiving no further attention. The organism was an anaerobic bacterium, thought to belong to the genus *Thiobacillus*, able to produce sulfide at the expense of sulfite and subsequently oxidize the sulfide to sulfur. Organic matter was required by the organism (7).

$$C_2H_5NaO_3 + 2 Na_2SO_3 = NaHCO_3 + 2 Na_2CO_3 + 2 H_2S \qquad (G)$$

$$2 H_2S + O_2 = 2 H_2O + S_2 \qquad (H)$$
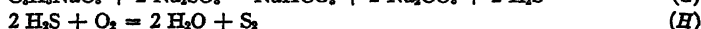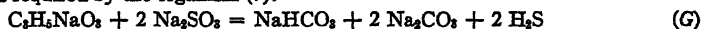
The evidence in the various reports clearly indicates that sulfur bacteria of the genus *Thiobacillus* are widely distributed. They occur in most soils, upon rocks, in salt water and fresh water, in sewage, and in mineral springs. Organisms capable of oxidizing thiosulfate may even be sufficiently abundant as laboratory contaminants to cause the decomposition of the thiosulfate solutions which are used as chemical reagents (37).

EXPERIMENTAL

For detecting the presence of organisms oxidizing the sulfur materials, the media shown in table 1 were used. The thiosulfate and bicarbonate, where used, were sterilized separately, as was also the ammonium sulfate for media II, III, and IV. The media were used in 100-cc. portions in 250-cc. Erlenmeyer flasks.

Soils were collected with aseptic precautions. Each of three flasks received 100-gm. portions of each soil. One portion was untreated; the second received 2 gm. sterile sulfur, and the third, 1 gm. sterile sodium thiosulfate. The moisture content was kept favorable during the 3 weeks of incubation at 28°C. Suspensions containing 0.5 gm. soil were then inoculated into flasks of thiosulfate and sulfur media.

Seven samples of soil (all black clay loams) were obtained from the fruit farm of the University of Minnesota. Nine other samples were obtained from Wis-

consin, numbers 8–13 being clay or clay loams and numbers 14–16 being sandy loams. These all supported field crops or grass. The soils were close to neutrality in reaction, varying between pH 6.1 and 7.1. As a result of incubation with sulfur the reactions dropped to pH 1.8–4.3. There was no appreciable change in reaction in any of the soils due to the presence of thiosulfate.

None of the soils caused growth, change in reaction, or sulfur oxidation in the acid sulfur solution medium (I). Enrichment of the soil with sulfur or thiosulfate previous to inoculation failed to effect the development of organisms in this medium even though the soil reaction had become very acid during the preliminary incubation. Oxidation of thiosulfate was rapid with most of the soils upon inoculation into medium II (table 2). The pretreatment with sulfur or thiosulfate failed to favor the process; in fact, somewhat slower oxidation was obtained from treated soils 1–7. The characteristics of growth were

TABLE 1

Media used for cultivating organisms upon sulfur materials

CONSTITUENTS	I	II	III	IV
	For Th. thiooxidans	For thiosulfate decomposers		
Distilled water..........	100.0 cc.	100.0 cc.	100.0 cc.	....
Tap water..............	....	....	....	100.0 cc.
Sulfur.................	1.0 gm.	....	....	....
$Na_2S_2O_3 \cdot 5H_2O$..........	....	0.5 gm.	0.5 gm.	1.0 gm.
$(NH_4)_2SO_4$..............	0.04 gm.	0.04 gm.	0.02 gm.	0.01 gm.
KH_2PO_4................	0.4 gm.	....	....	....
K_2HPO_4...	....	0.4 gm.	0.2 gm.	0.2 gm.
$CaCl_2$..................	0.025 gm.	0.025 gm.	0.025 gm.	0.01 gm.
$MgSO_4 \cdot 7H_2O$...........	0.05 gm.	0.05 gm.	0.02 gm.	0.01 gm.
$MnSO_4$.................	....	....	....	0.002 gm.
$FeSO_4$.................	0.001 gm.	0.001 gm.	0.001 gm.	0.002 gm.
$NaHCO_3$...............	....	....	0.05 gm.	....
pH....................	4.8	7.0	8.5	7.8

diverse. In some cases the medium was turbid; in others, sulfur precipitated on the liquid surface or upon the wall of the flasks; and in still other cultures, thin membranes, free from sulfur, appeared on the surface. Combinations of these effects were also noted. As growth progressed the reactions of the media became somewhat acid in all cases although varying in degree among the cultures. Transfers were made to fresh media, in which the thiosulfate was again rapidly oxidized. Some of the cultures were plated upon an agar medium containing thiosulfate, and colonies were recovered which in some cases were impregnated with sulfur and in other instances appeared small and moist like drops of dew. Almost invariably there were clear zones about the developing masses of bacteria, suggesting the formation of acid during oxidation of the thiosulfate. These cultures decomposed thiosulfate upon reinoculation into the solution medium.

Thirteen samples of soil materials were gathered from the farm of the N. J. Agricultural Experiment Station (see table 3) and inoculated, without preliminary treatment, into the acid sulfur solution medium. These soils varied considerably in texture, reaction, and vegetation. Only two of the samples gave growth and sulfur oxidation in medium I. One of these (soil 29) had

TABLE 2

Oxidation of thiosulfate in solution medium II following soil inoculation

PRETREATMENT OF SOIL	SOIL NUMBER	THIOSULFATE REMAINING IN 5 CC. OF MEDIUM			SOIL NUMBER	THIOSULFATE REMAINING IN 5 CC. OF MEDIUM		
		10 days	15 days	30 days		10 days	15 days	30 days
		mgm.	mgm.	mgm.		mgm.	mgm.	mgm.
Untreated	Control	19.2	19.2	21.3	Control	19.2	19.2	19.2
	1	0	0	0	8	13.8	....	0
	2	0	0	0	9	19.2	....	11.9
	3	0	0	0	10	2.1	....	0
	4	0	0	0	11	14.9	....	14.5
	5	0	0	0	12	0	....	0
	6	0	0	0	13	19.2	....	1.3
	7	0	0	0	14	19.2	....	17.7
					15	9.6	....	3.1
					16	9.6	....	1.9
2 per cent s	1	17.3	0	0	8	13.6	0	0
	2	16.0	18.0	18.0	9	14.9	14.1	14.9
	3	18.5	19.0	20.3	10	....	....	....
	4	18.0	19.2	20.9	11	13.0	0	0
	5	17.7	15.3	0	12	15.3	13.4	9.2
	6	0.4	0	0	13	16.4	13.6	9.8
	7	5.1	0	0	14	4.3	0	0
					15	15.5	15.1	16.3
					16	0	0	0
1 per cent $Na_2S_2O_3$	1	6.0	0	0	8	16.8	14.1	0
	2	18.1	18.7	18.3	9	18.7	19.2	22.4
	3	13.8	1.7	0	10	18.1	10.7	0
	4	14.9	0	0	11	17.5	15.8	16.8
	5	0	0	0	12	16.2	0	0
	6	12.8	0	0	13	14.5	8.2	0.6
	7	0	0	0	14	17.5	16.1	0
					15	18.1	17.6	16.8
					16	12.1	0	0

received a heavy application of sulfur and rock phosphate and had been inoculated with *Th. thiooxidans* about 10 years previous to the sampling. There still remained a considerable quantity of unoxidized sulfur. The extremely acid condition (pH 2.2) is clear evidence of extensive sulfur oxidation. Growth from this soil was active and typical for *Th. thiooxidans*. Evidently,

ROBERT L. STARKEY

TABLE 3

Decomposition of thiosulfate in solution media following soil inoculation

SOIL NUMBER	FIELD TREATMENT*	pH OF SOIL	THIOSULFATE			
			32 days after first addition		Second addition of thiosulfate 11 days after addition	
			Found	Decom-posed	Found	Decom-posed
			mgm.	*mgm.*	*mgm.*	*mgm.*
Blank	....	...	20.1	....	22.6	....
17	(7A) Untreated	4.7	16.6	3.5	15.0	7.6
			15.8	4.3	18.6	4.0
18	(7B) Lime	6.9	19.0	1.1	....	....
			15.8	4.3	17.8	4.8
19	(5A) Minerals, manure	5.8	1.6	18.5	0	22.6
			5.1	15.0	10.7	11.9
20	(5B) Minerals, manure, lime	7.1	0	20.1	23.3	0
			0.8	19.3	10.7	11.9
21	(9A) Minerals, NaNO₃	5.5	17.8	2.3	....	....
			18.2	1.9	....	....
22	(9B) Minerals, NaNO₃, lime	7.2	16.6	3.5	....	....
			0	20.1	21.7	0.9
23	(11A) Minerals, (NH₄)₂SO₄	4.1	18.6	1.5	....	....
			17.0	3.1	11.1	11.5
24	(11B) Minerals, (NH₄)₂SO₄, lime	6.0	15.0	5.1	5.9	16.7
			15.0	5.1	6.3	16.3
25	Vegetable crops, sandy loam	7.0	15.4	4.7	13.8	8.8
			15.8	4.3	20.5	2.1
26	Pasture, clay loam	7.0	15.8	4.3	15.8	6.8
			5.5	14.6	19.8	2.8
27	Hardwood forest, A₀ horizon	4.6	9.9	10.2	16.6	6.0
			17.8	2.3	....	....
28	Hardwood forest, A₁ horizon, clay	4.6	19.0	1.1	....	....
			19.0	1.1	....	....
29	Barren sulfur treated loam	2.2	....	....	....	....

* Soils 17–24 are from experimental plots receiving different fertilizer treatments for over 20 years. The soil is a sandy loam supporting field crops in rotation.

this organism will persist for a long time, if not indefinitely, in soils under natural conditions if elemental sulfur is available. The development of an extremely acid condition appears to have no injurious effect in soil. It has previously been found to exert very slight action in solution media (57).

The other sample which gave growth was number 27, which was composed of decomposing litter of a hardwood forest. Growth in this case was somewhat slower than by the usual cultures of *Th. thiooxidans* although the reaction was brought below pH 1.0 by growth in some of the flasks of medium I to which the culture was transferred. This culture was cultivated with monthly transfers for more than 2 years before being discarded.

These results again emphasize that *Th. thiooxidans* is not generally readily recovered from soils or other natural materials which are not supplied with elemental sulfur or similar inorganic sulfur materials.

Medium III which was used for these soils is similar in composition to that used by Beijerinck for *Th. thioparus* (7, 8). The soils were inoculated in duplicate flasks of medium. All of the soils decomposed some thiosulfate in 32 days, although the transformation was very meagre in soils 17, 18, 21, 23, and 28. These soils represent the most infertile ones of the group, some of which were quite acid in reaction. The most fertile soils, 19 and 20, decomposed the thiosulfate almost completely.

To a number of these cultures additions of sterile thiosulfate were made. The results after incubation for 11 additional days are given in the last two columns of table 3. In most cases decomposition continued. One culture each of soils 20 and 22 failed to develop, however; these cultures were the only ones that had decomposed the thiosulfate completely previous to the addition of more thiosulfate. It seems likely that in these instances the organisms had died as a result of exhaustion of the food material and the increase in acidity which generally accompanied extensive growth. After 10 days of further incubation, the thiosulfate had completely disappeared from all of the cultures except one each of soils 17, 20, 22, and 27.

In order to determine whether or not the initial reaction of the medium affected growth, media similar to medium III but adjusted over the range of pH 6.0 to 9.0 were prepared. Two of the neutral fertile soils, 20 and 25, were used. It is apparent from table 4 that the initial reaction exerted little effect on oxidation of thiosulfate. Organisms in soil 20 grew at all reactions and decomposed practically all of the thiosulfate at all reactions except pH 8.5. This exception appears to have no significance. With soil 25 decomposition was complete at both extremes of reaction. When further additions of thiosulfate were made to these solutions no growth occurred from cultures which had caused 100 per cent decomposition, but growth did develop from such cultures as from soil 20 of pH 8.0 and 9.0 which still contained some undecomposed thiosulfate at the time of the fresh addition. This suggests again that the bacteria tend to die out rapidly after the energy source is exhausted. In case the cultures are not transferred within a period of 2 or 3 weeks, the organisms

which cause abundant precipitation (probably *Th. thioparus*) are soon lost. Beijerinck noted that *Th. thioparus* died out in a short period on solid media (8).

Although soils seldom show the presence of organisms able to oxidize sulfur in acid media, they more frequently contain forms oxidizing sulfur to sulfate

TABLE 4

Decomposition of thiosulfate in media differing in initial reaction

		THIOSULFATE			
SOIL NUMBER	pH OF MEDIUM	12 days		28 days	
		Found	Decomposed	Found	Decomposed
		mgm.	*mgm.*	*mgm.*	*mgm.*
Control medium*	...	18.2	....	20.5	....
20	6.0	0	18.2	0	20.5
20	6.5	4.7	13.5	0	20.5
20	7.0	14.6	3.6	0	20.5
20	7.5	0	18.2	0	20.5
20	8.0	17.4	0.8	5.5	15.0
20	8.5	16.6	1.6	16.6	3.9
20	9.0	16.6	1.6	1.2	19.3
25	6.0	14.2	4.0	0	20.5
25	6.5	17.0	1.2	19.0	1.5
25	7.0	17.4	0.8	19.4	1.1
25	7.5	17.4	0.8	12.6	7.9
25	8.0	17.0	1.2	17.8	2.7
25	8.5	9.9	8.3	0	20.5
25	9.0	16.6	1.6	0	20.5

* Average of 7 flasks.

TABLE 5

Oxidation of elemental sulfur by crude cultures

SOIL SOURCE OF CULTURE	pH	TOTAL SULFATE-S IN 100 CC.
		mgm.
Control	9.0	14.9
20	5.8	80.3
20	5.6	118.9
25	5.8	32.3
25	5.2	84.9
26	6.0	31.7
26	6.0	52.9

in somewhat alkaline solutions. Crude cultures from three of the soils mentioned in table 3 were inoculated into an inorganic sulfur medium similar to medium I but containing 1 per cent $CaCO_3$. The results of table 5 indicate fairly active transformation of sulfur with appreciable increase in acidity.

Growth was accompanied by the development of a light turbidity of the medium. These cultures gave similar changes upon repeated transfers. No isolations of pure cultures were attempted with these solutions. It is reasonable to suppose that the growth was caused by organisms similar to *Th. thioparus*, since such cultures were subsequently isolated from these soils.

For the following studies medium IV was used. For a solid medium 1.5 per cent agar was added to this solution. Many of the cultures which effected appreciable oxidation of thiosulfate were used for the isolation of pure cultures. For this purpose nutrient and thiosulfate agar media were used.

The crude cultures showed a predominance of Gram-negative, small, rod-shaped or oval bacteria, some of which were motile. A great variety of protozoa (amoebae, flagellates, and ciliates) were encountered, particularly in the most active cultures. The bacteria were frequently found in the midst of precipitated sulfur, some of which was granular or crystalline but mostly in the amorphous state as highly refractive globules frequently having a diameter 10 or more times the longer dimension of the bacteria.

Only cultures from the New Jersey soils were used in the isolation studies. As a result of repeated plating, isolation, and culture on the solution medium a considerable number of pure cultures were obtained. Many of these failed to decompose thiosulfate and were discarded. The thiosulfate oxidizers were cultured on some of the customary laboratory media in order to determine how many of the cultures were distinct species. Morphological characteristics were also determined. More than 100 cultures were thus studied, and these yielded but three which could be considered as distinct, typical species.

Culture A

Thiosulfate solution. Uniformly turbid with no pellicle or material clinging to walls of the flask. Some whitish sediment, which is loose and unconsolidated, generally appears together with a thin incomplete membrane on the bottom of the flask. The reaction becomes acid within a few days, changing from an initial pH 7.8 to 5.8. This change is associated with the decomposition of a comparatively small quantity of thiosulfate. Further growth is probably prevented by the acidity, since the reaction seldom drops below pH 5.8. (See plate 2, fig. 11c.)

Sulfur solution medium of slightly alkaline reaction. No growth.

Nutrient agar slant. Soft, somewhat ropy growth, fairly abundant, raised, shining, and moderately spreading. Whitish in reflected light, brownish opalescence in transmitted light.

Nutrient agar stab. Fairly abundant mass of white mucoid material on the surface about the point of the stab. During the first few days not extending more than 1 cm. deep, gradually penetrating deeper, reaching the bottom of the tube. Growth white to cream colored confined close to the line of inoculation.

Nutrient agar plate. Growth slow. In 4 to 5 days surface colonies up to

1 mm. in diameter, round, raised, moist, and colorless, similar to drops of thin starch paste. Deep colonies tiny, lens shaped up to about 0.5 mm. in longer dimension in 5 days. (See plate 2, fig. 9.)

Thiosulfate agar slant. Very thin and practically colorless, not penetrating below the agar surface. In most cases accompanied by some sulfur which is precipitated as a white frosty film on the surface of the slant.

Thiosulfate agar stab. No appreciable growth on the surface. Very thin growth and shadowy in outline extending slightly from the line of the stab, like a thin veil.

Thiosulfate agar plate. Colonies develop slowly, becoming white from sulfur which is precipitated. Sub-colonies are lens shaped or round from pin-point size up to 1 mm. in long dimension in 1 or 2 weeks; surface colonies small, round, and moist reaching 1 to 1.5 mm. in diameter. Crystals of calcium sulfate appear throughout the agar as growth progresses. (See plate 1, fig. 4a.)

Nutrient solution. Slight turbidity. Tendency for formation of pellicle, which becomes complete and gelatinous. Most characteristic is the formation of a long streamer-like network extending from the surface to the bottom of the tube and forming some sediment.

Potato slant. Limited development of cream colored growth, moist, shining, and slightly brown.

Litmus milk. Slow development of slight alkalinity.

Gelatin stab. Mucoid growth at point of inoculation or slightly spreading in a circular zone at the surface. Very thin growth below the surface. No liquefaction during first week. Slight saccate liquefaction in a few weeks.

Morphology. Small, Gram-negative, non-sporulating, non-motile, short rods or coccoid cells varying in size from 0.4–0.8 x 0.6–1.8 μ. Average size 0.6 x 1.2 μ. The organism takes the common stains readily. (See plate 1, fig. 4b.)

Source. This organism was recovered from soils 20 and 25. The cultures used for physiological studies came from soil 20.

Culture B

Thiosulfate solution. Comparatively slight evidence of growth. Occasionally light turbidity during first few days. No pellicle. Generally the small amount of precipitate common to the medium becomes drawn together in flocculent aggregates. Occasionally a small amount of elemental sulfur appears as a result of secondary reactions. Slow decomposition of thiosulfate is accompanied by an increase in alkalinity, which may change from pH 7.8 initially to over pH 9.0.

Sulfur solution medium. No growth.

Nutrient agar slant. Smooth, thin, spreading, fairly abundant growth, soft and buttery in consistency. White to gray in reflected light, light brown in transmitted light. Young cultures slightly fluorescent.

Nutrient agar stab. Smooth, spreading growth on surface with dense center and serrated edges. Develops well into the medium as granular growth confined closely to the line of the stab.

Nutrient agar plate. Colonies circular, fairly large on surface (5 mm. in diameter in 2 days). Flat with raised center, surface granular. Deep colonies, tiny lens shaped (about 1 mm. in long dimension). Young cultures fluorescent. (See plate 2, fig. 7.)

Thiosulfate agar slant. Thin growth spreading over surface as tiny droplets, some cases scarcely visible, in others fairly heavy and white as a result of appearance of elemental sulfur. Growth penetrates the agar and produces a fine granular opacity beneath the surface of the slant, particularly pronounced at the base.

Thiosulfate agar stab. Little or no growth on surface, except occasional droplets. Grows well to about 1 cm. deep with granular particles spreading from the line of stab. Growth below this region thin and limited.

Thiosulfate agar plate. Colonies thin and diffuse, somewhat denser about the center. Become as large as 5 mm. in diameter in 3 days. Tendency to grow into the agar. When reaching the surface, the center of the colony appears as a tiny moist drop. (See plate 1, fig. 1a.)

Nutrient solution. Thin fragile pellicle which settles to bottom. Dense uniform turbidity. Abundant sediment.

Potato slant. Fairly abundant, somewhat spreading mucoid, cream colored growth turning brown.

Litmus milk. Turns strongly alkaline with no digestion.

Gelatin stab. No liquefaction in 4 weeks. Uniform gray growth over surface. Scant development along the line of stab.

Morphology. Small coccoid, Gram-negative, non-sporulating cells frequently occurring in pairs appearing like rods with terminal granules. Actively motile. Vary in size from 0.3–0.7 x 0.4–1.2 μ. Average size 0.5 x 0.7 μ. Readily stained. (See plate 1, fig. 1b.)

Source. Recovered from soils 20, 25, and 26, cultures from the first two sources were used for physiological studies.

Culture C

Thiosulfate solution. Growth is very rapid, becoming turbid in 24 hours as a result of initial precipitation of sulfur. As growth proceeds the sulfur either accumulates as a complete membrane mixed with bacterial cells on the surface or is deposited on the walls and bottom of the flask. The medium remains somewhat turbid. Complete decomposition of the thiosulfate occurs within a week or 10 days, and the reaction becomes acid, frequently dropping from the initial pH 7.8 to as low as pH 4.0–4.5. Cultures must be frequently transferred (every 2 or 3 weeks). (See plate 2, fig. 11b.)

Sulfur solution medium. Characteristics uncertain. Oxidizes the sulfur, if at all, much more slowly than thiosulfate. Forms some turbidity. The reaction becomes acid.

Thiosulfate agar slant. Thin, veil-like droplet film developing on the surface. The small masses of cells become brown in old cultures. Throughout the agar, tiny colonies develop, which together with small aggregates of pre-

cipitated sulfur and crystals of various sulfates give a uniform milky appearance to the entire agar contents. The organism dies out upon such media if not frequently transferred (every 1 to 3 weeks). Growth is much the same as for *Th. thiooxidans* on thiosulfate agar.

Thiosulfate agar stab. On the surface, a thin film of droplets develops about the point of inoculation. These droplets become coated with sulfur. Upon aging they turn brown. Growth follows thé stab to a depth or 2 or 3 cm. forming a thin veil showing some precipitation of sulfur.

Thiosulfate agar plate. Colonies develop slowly. When heavily inoculated, only microscopic colonies appear, which cause the entire agar plate to become cloudy with precipitated sulfur interspersed with crystals of calcium sulfate with clear zones about the colonies. When few or scattered, the colonies are larger, moist, and soft. Surface colonies are circular from 1 to 2 mm. in diameter in a period of several days to one month; sub-colonies are lens shaped up to 1 mm. in long dimension (plate 1, fig. 5a). Colonies first appear white with precipitated sulfur and later become brown but are still impregnated with sulfur, which appears microscopically as variously sized, highly refractive, yellow globules, most of which vary in size between 1 and 10 μ in diameter. (See plate 1, fig. 6.)

No growth on organic media.

Morphology. Tiny coccoid to oval, Gram-negative, non-sporulating cells. Pure cultures non-motile. Frequently encountered mixed with organism B, which is not readily distinguishable morphologically except by its active motility. Varies in size· from 0.3–0.7 x 0.4–0.9 μ. Average size 0.5 x 0.7 μ. Readily stained. (See plate 1, fig. 5b.)

Source. Recovered from soils 20, 22, 25. Culture used for physiological studies obtained from soil 25.

Culture C is hardly distinguishable morphologically from *Th. thiooxidans*, which measures 0.4–0.6 x 0.5–0.9 μ with average of 0.5 x 0.8 μ. *Th. thiooxidans* is, however, actively motile by means of a single polar flagellum which has a ribbon-like appearance and may be several times the length of the cell. (See plate 2, fig. 10.) It was stated in earlier reports that *Th. thiooxidans* is also Gram-positive (69). More recent repeated tests have shown that the organism is Gram-negative. It cannot be distinguished from other members of the genus *Thiobacillus* by the Gram reaction [see in this connection (15, 16, 49)].

Culture C appears to be a strict autotroph and in this and other respects shows physiological characteristics practically identical with *Th. thioparus* and may be considered as this species, at least tentatively. *Th. thioparus* was reported by Beijerinck (8) as being actively motile and having somewhat larger dimensions (3 x 0.5 μ).

Culture A is a facultative autotroph which does not appear to have been described previously. By reason of its physiological behavior it belongs to the genus *Thiobacillus*. The name *Thiobacillus novellus* nov. sp. is herewith given to this organism.

Culture B is very similar to the cultures of Trautwein in its activity upon the thiosulfate medium and development upon the organic media (62, 63).   Trautwein's cultures were named *Th. trautweinii* by Bergey (9).   In view of the fact that it was found in studies to be reported subsequently that neither this organism nor culture B are autotrophic, their inclusion in the genus *Thiobacillus* is unwarranted.   Culture B is somewhat smaller in size than *Th. trautweinii*. Trautwein gave the average dimensions of his organisms as 0.4 x 1.3 μ.   Measurements of his cultures made in this laboratory average 0.6 x 1.6 μ.   Trautwein's cultures T and K have been preserved here for about 10 years and have been used in various comparative tests with the three cultures already mentioned.   These organisms have morphological and colony characteristics very similar to those of culture B (plate 1, fig. 2a, 2b, 3a, 3b; plate 2, fig. 8).   A third culture of Trautwein (W) was lost from our collection of cultures.

The characteristics of the various organisms when developing upon the thiosulfate medium are compared in table 6.

TABLE 6

Development of organisms in thiosulfate solution

CULTURE	AGE	pH*	TITRATION OF 5 CC. WITH 0.01 N IODINE	GROWTH CHARACTERISTICS
	days			
Control..............	14	7.8	20.5	—
B...................	14	8.8	15.9	Slight flocculent sediment
Trautwein (T).........	14	8.2	16.3	Slight flocculent sediment
Trautwein (K).........	14	8.8	15.0	Slight flocculent sediment
A (*Th. novellus*)........	14	5.6	17.5	Turbid. Slight bottom film
C (*Th. thioparus*)......	14	4.6	0	Heavy precipitation of sulfur
Th. thiooxidans.........	14	1.4	0	Turbid

* The initial reaction for *Th. thiooxidans* was pH 6.0, since it fails to develop in solution media at alkaline reactions.

It seems evident that the transformation of thiosulfate by these organisms must involve a variety of reactions, since both the visual characteristics of growth and the changes in pH are distinctly different for the various species. A discussion of these differences and of the influence of environmental conditions on the course of the processes is reserved for subsequent reports.

SUMMARY AND CONCLUSIONS

Only 2 of the 29 soils examined indicated the presence of autotrophic bacteria oxidizing sulfur under acid conditions, and 1 of these soils had previously been inoculated with *Th. thiooxidans* and had received an application of sulfur. Organisms able to effect the oxidation of sulfur in somewhat alkaline media are more readily detected.   In all of the 28 soils, organisms were found which decomposed thiosulfate in mineral solution.   The rapidity and extent of oxidation varied with the soil.   Decomposition of thiosulfate by crude cultures took place in mineral media over a wide range of reaction (pH 6.0–9.0).

Three cultures which were physiologically distinct from one another were obtained from the soils:

1. One of the cultures (A), which is a facultative autotroph, seems to be a new species and is designated by the name *Thiobacillus novellus* nov.´sp. It oxidizes thiosulfate with acid formation and fails to oxidize elemental sulfur. It is a small, Gram-negative, non-motile, non-sporulating rod $0.6 \times 1.2\mu$ in size. It grows well on both organic media and mineral media containing thiosulfate.

2. Culture B is closely related to cultures of Trautwein named *Th. trautweinii*, Bergey et al. In the oxidation of thiosulfate by these bacteria the pH of the media increases. They make little visible evidence of growth in mineral thiosulfate solutions and grow on a variety of organic media. These cultures are not autotrophic and do not belong to the genus *Thiobacillus*.

3. Culture C is identical with or at least closely related to *Th. thioparus*, Beij. During oxidation of thiosulfate, the medium becomes acid and elemental sulfur is precipitated. This is a strict autotrophic bacterium, a small, non-sporulating, Gram-negative, non-motile rod $0.5 \times 0.7\mu$ in size.

Some of the cultural and morphological characteristics of these organisms are presented and compared with *Th. thiooxidans*, Waks. and Joffe, which is also a Gram-negative tiny rod 0.5×0.8 μ in size.

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PLATE 1

COLONY DEVELOPMENT DURING 12 DAYS ON THIOSULFATE AGAR. 1.8 ×

FIG. 1a. Culture B.

FIG. 2a. Culture T (*Th. trautweinii*).

FIG. 3a. Culture K (*Th. trautweinii*).

FIG. 4a. Culture A (*Th. novellus*).

FIG. 5a. Culture C (*Th. thioparus*).

PHOTOMICROGRAPHS OF CELLS FROM THIOSULFATE AGAR CULTURES, 5 DAYS OLD. 1000 ×

FIG. 1b. Culture B.

FIG. 2b. Culture T (*Th. trautweinii*).

FIG. 3b. Culture K (*Th. trautweinii*).

FIG. 4b. Culture A (*Th. novellus*).

FIG. 5b. Culture C (*Th. thioparus*).

FIG. 6. Globules of sulfur from a colony of culture C developing upon thiosulfate agar. 468 ×.
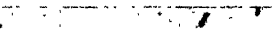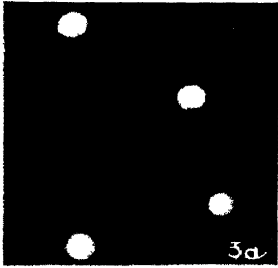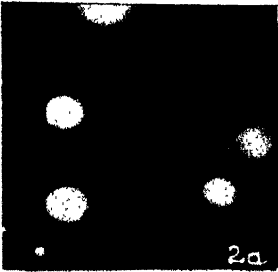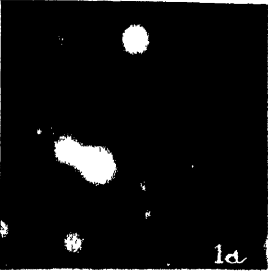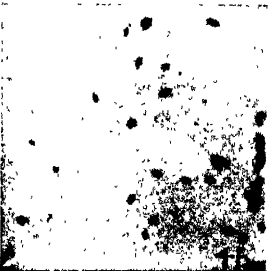
1b   2b   3b

PLATE 2

COLONY DEVELOPMENT DURING 5 DAYS ON NUTRIENT AGAR.   1.8 ×

FIG. 7. Culture B.

FIG. 8. Culture T (*Th. trautweinii*).

FIG. 9. Culture A (*Th. novellus*).

FIG. 10. Photomicrograph of *Th. thiooxidans* showing flagellation.   2160 ×.

FIG. 11. *a*. Uninoculated thiosulfate solution medium.

b. Thiosulfate solution medium supporting growth of culture C (*Th. thioparus*).   General turbidity with sulfur precipitation in culture 7 days old.

c. Thiosulfate solution medium supporting growth of culture A (*Th. novellus*).   Uniformly turbid solution of culture 7 days old.
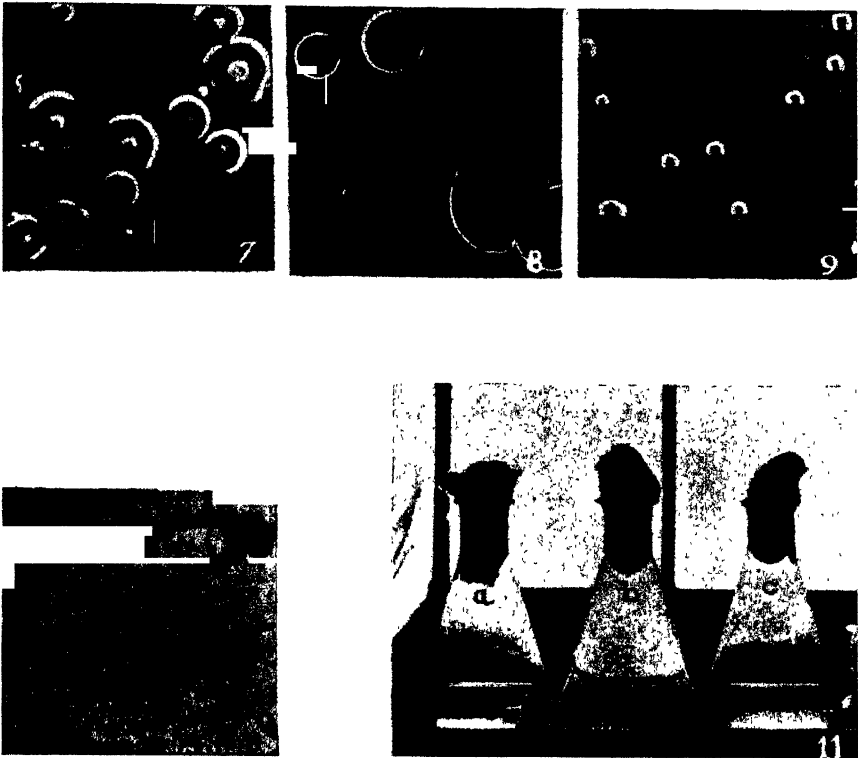
THE NOMENCLATURE OF THE COWPEA GROUP OF ROOT-NODULE BACTERIA[1]

R. H. WALKER AND P. E. BROWN

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From the time the root-nodule bacteria of leguminous plants were first studied there has been considerable interest in the differentiation of species. At first it was believed that a single species of bacteria was responsible for the production of nodules on all legume plants. As the study of these organisms continued, however, it was learned that the organisms responsible for the production of nodules on some legumes are different in certain morphological or physiological characteristics from those producing nodules on other legumes. Hence, there has arisen the problem of the differentiation of species.

In a number of publications it has been suggested that the organisms producing nodules on certain plants be recognized as constituting separate species. These reports have been adequately reviewed by Buchanan (2) and by Baldwin and Fred (1), the latter investigators having made, in addition, the definite suggestion that the organisms of certain cross-inoculation groups be considered as constituting separate species.

Because of a lack of information concerning the bacteria of certain legumes or groups of legumes, the classification and nomenclature of the root-nodule bacteria have not yet been completed. Perhaps the outstanding example of this is in the case of the organisms producing nodules on cowpea and other plants of the same cross-inoculation group.

There has been some uncertainty as to whether or not the cowpea root-nodule bacteria should be classified with the soybean bacteria. It was early recognized by Kirchner (5) that the soybean bacteria are different from most of the other root-nodule organisms. He suggested that the soybean organisms be placed in a separate species, for which he proposed the specific name *japonicum*, which is now recognized as valid and is in rather general use by American bacteriologists. Numerous experiences of a practical nature have shown the desirability of inoculating soybean seed with organisms isolated directly from soybean root-nodules. Furthermore, numerous controlled cross-inoculation experiments have indicated that the soybean bacteria infect only soybean plants and that root-nodule organisms from other plants do not infect soybeans. As a result the belief that the soybean bacteria are distinctly different from the other root-nodule organisms, including those of cowpeas, has become rather general.

What may seem as an apparent contradiction to the generally accepted belief concerning the failure of soybean and cowpea organisms to cross-inoculate are the results reported in recent years by Leonard (6), Sears and Carroll (7), Hansen and Tanner (4), and Carroll (3). These investigators found that certain strains of cowpea bacteria are capable of producing nodules on soybeans and that certain strains of soybean bacteria are capable of producing nodules on cowpeas. The interchangeability has not been considered perfect in all cases, however, as in certain cases where soybean organisms were found to produce nodules on cowpeas, it was observed that the nodules were not typical of those produced on cowpeas by cowpea organisms. Furthermore, not all strains of the cowpea organisms nor of the soybean organisms tested were found to cross-inoculate.

221

This situation has led to a little uncertainty as to the proper classification of the organisms of these two cross-inoculation groups. Inasmuch as the principal criterion for species differentiation within the genus Rhizobium has been the ability to inoculate plants, it has not been known definitely whether the organisms of the soybean and cowpea root-nodules should be considered as belonging to the same or to different cross-inoculation groups.

In order to obtain additional information on this problem, the following cross-inoculation experiments with a number of strains of soybean and cowpea root-nodule bacteria were conducted.

EXPERIMENTAL

Five strains of organisms that were at one time isolated from cowpea root-nodules and fifteen strains similarly isolated from soybean root-nodules were tested for their ability to cross-inoculate. In the first experiment the five

TABLE 1

The formation of nodules on cowpea and soybean roots by five strains of cowpea root-nodule bacteria

STRAINS OF COWPEA BACTERIA	NODULATION OF COWPEA PLANTS[*]		NODULATION OF SOYBEAN PLANTS	
	First	Second	First	Second
603	+	+	−	−
605	+	+	−	−
607	+	+	−	−
608	+	+	−	−
609	+	+	−	−
Control	−	−	−	−
Control	−	−	−	−

* + indicates that nodules were produced; − indicates that no nodules were produced.

strains of cowpea organisms were used to inoculate both soybean and cowpea seeds. In the second experiment the fifteen strains of soybean bacteria were similarly used to inoculate both kinds of seed.

The seeds were first sterilized with hydrogen peroxide and then inoculated with the various strains of bacteria. After being inoculated the seeds were planted in sterile sand, where they were permitted to grow in the greenhouse for 3 weeks. At that time the plants were removed from the sand cultures and observed for nodulation of the roots. The results of the two tests are reported in tables 1 and 2.

Quadruplicate controls of uninoculated seeds were carried along in each test, and in no instance was there any sign of nodule development on any of the control plants. Furthermore, there was good agreement of duplicate cultures of the inoculated plants, and in no case did there appear to be any serious difficulty in determining whether or not nodules had been produced.

The data show rather definitely that, whereas all the cowpea bacteria produced nodules on cowpea roots, none of them produced nodules on soybean roots. On the other hand, whereas all of the soybean bacteria produced nodules on soybean plants, eight of them also produced nodules on cowpea plants. Three of the soybean cultures definitely failed to produce nodules on cowpeas, and four others each failed to produce nodules in one of the duplicate tests, but produced nodules in the other test in each case.

TABLE 2

The formation of nodules on cowpea and soybean roots by fifteen strains of soybean root-nodule bacteria

STRAINS OF SOYBEAN BACTERIA	NODULATION OF COWPEA PLANTS*		NODULATION OF SOYBEAN PLANTS	
	First	Second	First	Second
401	+	−	+	+
402	+	+	+	+
403	+	−	+	+
404	−	−	+	+
405	−	−	+	+
406	+	+	+	+·
407	+	+	+	+
408	− .	−	+	+
410	+	+	+	+
411	+	−	+	+
412	+	−	+	+
413	+	+	+	+
414	+	+	+	+
415	+	+	+	+
416	+	+	+	+
Control	−	−	−	−
Control	−	−	−	−

* + indicates that nodules were produced; − indicates that no nodules were produced.

DISCUSSION

The results, in general, are in accord with those reported by Leonard (6), Sears and Carroll (7), Hansen and Tanner (4), and Carroll (3), who obtained cross-inoculation with certain strains of the cowpea and soybean bacteria, but not with other strains. Results like those obtained in the test with the cowpea bacteria lead one to believe that the organisms that infect these two legumes are distinctly different. But the results of the tests with the soybean bacteria indicate that there is at least some relation between the two organisms and their abilities to infect plants. This naturally leads to the question of the species relationship of the two organisms.

In view of the results obtained by the several investigators of this problem, it seems logical that the bacteria producing nodules on the roots of soybeans

and cowpeas should be considered as belonging to the same species, instead of constituting separate species as they have been considered rather generally in the past. Carroll was undoubtedly of this opinion when he grouped the soybean bacteria in the cowpea cross-inoculation group. Further, it is assumed that Baldwin and Fred (1) were of somewhat the same opinion when they failed to give the organisms of the cowpea group a specific designation in their paper on the nomenclature of the root-nodule bacteria. If the cowpea and soybean organisms are to be considered as belonging to the same species, then according to the rule of priority in botanical nomenclature, they would be designated by the specific name proposed for the soybean organism by Kirchner in 1895 and now in general use in bacteriological literature. Thus the generic and specific name for this group would be *Rhizobium japonicum*.

The observed irregularities in the interchangeability of organisms to the various hosts within the cross-inoculation group may well be interpreted as being due to biologic variation of the strains of bacteria within the species. Variability in various physiological and serological characters among strains within species of Rhizobium has been quite definitely shown, and it is just as reasonable to assume that there may be a similar variability in the physiology of the organisms with respect to the interchangeability of hosts within a cross-inoculation group. As Carroll has pointed out, it is quite reasonable to assume that there may be some variability or irregularities in the infection of plants in a group of legumes as large and as variable as that of the cowpea group, now consisting of 17 known genera and 33 species of legumes. On the other hand, where the cross-inoculation groups are made up of a small number of closely related legumes such as in the alfalfa and clover groups, it is only natural that a greater uniformity of inoculation ability would be observed.

CONCLUSIONS

In view of the fact that certain strains of soybean bacteria have been found capable of producing nodules on cowpea roots, and that certain strains of cowpea bacteria have been found capable, by other investigators, of producing nodules on soybean roots; it is suggested that the root-nodule bacteria of soybeans and cowpeas be brought together in the one species now generally designated *Rhizobium japonicum* (Kirchner) Baldwin and Fred.

Different strains of this species of Rhizobium exhibiting difference in their abilities to produce nodules on certain plants within the cross-inoculation group may well be considered as biological variations within the species.

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A COMPARATIVE STUDY OF THE BACTERIAL FLORA OF WIND-BLOWN SOIL: IV. SHACKLEFORD BANK, NORTH CAROLINA

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An article by MacCarthy on the "Beach sands of the Atlantic Coast" (2) suggested to the writer that the high $CaCO_3$ content of the sand in the southern part of this coast might yield an interesting bacterial flora; consequently, on March 30, 1931, sand was collected from Shackleford Bank, N. C. for this study.[1]

METHODS AND MATERIALS

The methods and materials used were in general the same as those previously described (5, 6, 7). The sand was collected at three levels, 6, 12, and 24 inches, from the outer face of the Bank, about 100 feet from the tide (approximately half tide) and about 5 feet from the base of the slope. It was brought to Wellesley as rapidly as possible by motor and train and plated in the same manner as in previous work. The indicator media varied somewhat from those used in the previous studies. When the reaction in glucose gelatin was uncertain it was checked in glucose broth or agar. The latter in some cases was made with yeast extract instead of the usual beef-peptone base. Glucose positives were inoculated into sucrose broth or agar, the latter also made with yeast extract. Litmus lactose agar was used for the lactose fermentation test. Nitrate broth was used as formerly.

The presentation of results has been delayed because of the hope that another study of a similar soil might be made, but as this seems impracticable at present the following brief report is presented.

RESULTS

The average annual precipitation for the 5 years previous to the collection was 54.83 inches, with a variation of 40.70–73.26 inches, and the average annual mean temperature for this period was 63.96°F. with a variation of 13°F.–96°F. (8).

Analysis of soil

The high CO_3 content noted by MacCarthy (3) for a point $\frac{1}{4}$ mile northwest of Fort Macon, Beaufort, N. C., which is directly across the inlet from the

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[1] The collection was made possible by the courtesy of Dr. S. Hildebrand of the U. S. Bureau of Fisheries Laboratory, at Beaufort, N. C., to whom I wish to express my thanks.

place at which the present collection was made, is "7.75 percentage loss by leaching." This was obtained with HCl, whereas the present data were obtained from water-soluble salts only.

TABLE 1
Physical characters of soil

DEPTH	SIZES OF SOIL PARTICLES				WATER RELATIONS				COMBUS-TIBLE MATERIAL
	More than 1 mm.	1 to 0.5 mm.	0.5 to 0.1 mm.	Less than 0.1 mm.	Water content	Water capacity	Optimum* water content	Relative† water content	
inches	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
6	3.3	18.7	45.2	32.9	2.01	21.00	14.70	9.57	0.80
12	0.2	12.3	62.2	25.0	1.04	23.00	16.10	4.52	0.30
24	0.6	5.0	45.9	48.4	3.89	25.00	17.50	15.56	0.25
All	1.4	12.0	51.1	35.4	2.31	23.00	16.10	10.04	0.45

* 70 per cent of capacity.
† Percentage of capacity present.

TABLE 2
Chemical character of soil*

DEPTH	REACTION	TOTAL SOLUBLE SALTS	CO$_3$	SO$_4$	Cl	NO$_3$, etc.
inches	*pH*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
6	9.0	0.00918	0.00579	0.000834	0.00054±	0.00188
12	9.2	0.00992	0.00626	0.001803	0.00058±	0.00143
24	9.2	0.01294	0.00549	0.002188	0.00057±	0.00513
All	9.0–9.2	0.01068	0.00585	0.001608	0.00056±	0.00281

* The chemical analysis, except for the pH values, was made by Miss Miriam Dice, who used the same methods as she (5, 6) used in two of the previous analyses.

TABLE 3
Average numbers of organisms present in a gram of fresh soil

DEPTH	NUMBER OF PLATES COUNTED	TOTAL COUNT	BACTERIA OR YEAST	ACTINOMYCETES	FUNGI
inches			*per cent*	*per cent*	*per cent*
6	12	26,084	99.69	0.00	0.31
12	12	18,750	94.70	4.44	0.85
24	12	134,166	99.62	0.18	0.18
All	36	59,666	99.12	0.61	0.27

The high actual and relative water content of the 24-inch layer as compared with the percentages for the 12-inch layer possibly may account for the difference in the numbers and proportions of the organisms in the two. Cobb (1) found water to be the limiting factor in forest soils and cites numerous references for (and also against) this relationship.

One tube (culture 24–20) gave a basic reaction in litmus lactose agar but contained gas bubbles. Three repetitions of the test gave the same result, after which the culture was lost and no further work could be done. No gas appeared in glucose or sucrose and no other culture produced gas in any sugar. According to Merrill (4), only members of the genus Mycobacterium are able completely to ferment sugars to CO_2 and H_2O without the formation of organic acids. Culture 24–20 appeared to do this in lactose only. One difficulty, however, lies in the fact that Mycobacteria are strictly aerobic and unable to utilize lactose, whereas this culture showed bubbles of gas throughout the lactose agar.

TABLE 4

Colors of colonies on representative plates and cultures picked for study

GROUP	TOTAL NUMBER	WHITE	YELLOW	ORANGE	PINK	FLUORES-CENT
		per cent	per cent	per cent	per cent	per cent
Colonies......................	1,210	83.17	8.22	0.49	0.70	7.42
Cultures.....................	119	51.30	22.70	21.00	0.80	4.20

TABLE 5

Morphology and physiology of the organisms studied

DEPTH	TOTAL NUMBER	COCCUS OR COCCOID	PLEOMORPHIC	RODS		GRAM'S*		FERMENTERS			DIGESTORS		NO₂ REDUCERS†	
				Non-spore	Spore	Positive	Negative	Glucose	Sucrose	Lactose	Gelatin	Casein	Positive	Negative
inches		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
6	40	10.0	35.0	55.0	0.0	37.5	55.0	27.5	20.0	0.0	45.0	26.3‡	45.0	37.5
12	39	51.3	30.8	28.2	2.6	15.4	74.4	17.9	30.8	2.6	69.2	28.2	41.2	56.4
24	40	32.5	25.0	42.5	0.0	17.5	72.5	20.0	12.5	2.5	45.0	25.0	47.5	50.0
All	119	31.1	26.9	41.2	0.8	23.6	67.2	26.5	16.8	1.7	52.9	26.5	44.6	47.9

* Others were variable.

† The remaining percentage did not grow.

‡ Two cultures died before the test.

In regard to the single spore-former found the writer does not wish to imply that this, or a similar soil, is almost wholly lacking in spore-formers. Another analysis might yield a different result. The fact remains, however, that of 119 cultures picked from plates in an effort to get as many kinds of colonies as possible for study, only one form showed spores when cultured and stained according to the usual technique. In the previous studies, made as far as the author is aware in exactly the same way, 42.60 per cent (5), 22.47 per cent, and 19.67 per cent (6) of the cultures developed spores. An effort was made to check the heat resistance by exposing 25 of the 6-inch cultures to 80°C. for 10

minutes. After 2 days' incubation at 28°C. those that appeared cloudy were transferred to broth. Twenty of these were definitely negative, but five showed by growth that they had resisted the heat. Four of the five were pleomorphic forms, one gram-negative, and three gram-positive. This would suggest Mycobacterium, with one gram stain an error. The fifth form was a gram-negative short rod, probably a thermophile. It would be very desirable to do further work along this line.

Culture groups

The most conspicuous and homogeneous group was composed of 23 pale orange, or cream-colored cultures, oval-pleomorphic in form, gram-positive, non-fermenting, non-digesting, but strongly reducing organisms. A second fairly homogeneous group was composed of 36 pale yellow, white, or fluorescent cocci, or very short rods. These were gram-negative liquefiers which did not ferment sugars or, usually, reduce nitrates. The remaining 60 forms arranged themselves in small groups with various combinations of cultural characters.

Comparison of the north and south Atlantic soils

Although the soil of the north Atlantic coast was much coarser in quality than that of the south Atlantic coast, the water relations in the two were very similar. The southern soil was much more alkaline and contained larger amounts of soluble salts, but the water-soluble carbonate content was not very large. As water-soluble analysis had been used in previous studies, acid treatment for total carbonates would not have yielded comparable results.

A lower total number of organisms in the southern soil was composed almost wholly of bacteria, or yeasts, whereas the percentages of actinomycetes and fungi were much higher in the northern soil. When the previous discussion on the Arizona soil (5) regarding percentages of actinomycetes in soils is recalled, the distribution of these organisms in the soils in question is not very enlightening. Aridity cannot be the prime factor, as the water relations in the two soils were very similar. Nor can alkalinity account for the larger number in the northern region, since the southern soil had a higher pH. '

The percentages of colored colonies found on plates were not widely different in the two soils, the larger percentage of fluorescent forms in the northern soil being the most outstanding difference. The cultures from the southern soil had the larger percentage of yellow and orange and the smaller percentage of pink forms. The northern soil cultures had a small percentage of brown and no fluorescent forms, but the reverse was true of the southern cultures.

Morphologically and culturally the forms studied were rather similar, the greatest variation noted being the almost total absence of spore-formers in the southern soil, as against 19.67 per cent in the northern soil. Casein digestion and nitrate reduction were more pronounced in the southern soil.

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A COMPARATIVE STUDY OF THE BACTERIAL FLORA OF WIND-BLOWN SOIL: V. MONTEREY PENINSULA, CALIFORNIA

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This paper is the fifth in a series of brief surveys made in the attempt to compare bacteriologically similar soils under various climatic conditions (1). The work was done at the Hopkins Marine Station of Stanford University, at Pacific Grove, California.[1]

METHODS AND MATERIALS

The methods were in general those used in the previous work. Because the dunes all along the coast of the Peninsula are constantly traversed by people and animals it was impossible to find a region not influenced by human contact. The best that could be done was to select places for collection as far as possible from visible tracks. The first collection was made on July 8, 1932 from the smooth south-by-west face of a low dune south of Asilomar, and approximately 100 yards from the ocean. Collections were made from three levels, as formerly; namely, 6, 12, and 24 inches. On October 10, after 3 months without rain, a second collection was made approximately 1 mile farther south. The 6-inch layer in this case was very dry, and the level could not be accurately maintained. An analysis showed that this upper section contained 0.12 per cent water; the 12-inch layer, 1.14 per cent; and the 24-inch layer, 2.08 per cent. No other chemical or physical characters were studied for this collection. The sand in both cases was brought immediately to the laboratory and plated as usual in nutrose agar. For isolation of pure cultures yeast-autolysate agar, having the following composition was used: K_2HPO_4, 1 gm.; $MgSO_4$, 5 gm.; agar, 20 gm.; tap water, 900 cc.; yeast autolysate, 100 cc. From the plates of both collections colonies were fished for further study. Morphological and physiological studies were made. Glucose gelatin, sucrose, lactose, nitrate, and nutrose agar were used for the tests.

CLIMATE

The climatological data from Del Monte (2) are used for 1929, 1930, and 1931. As there were no reports for that station for 1927 and 1928 the figures for Salinas are substituted, since the data from that station agreed very closely

with those for Del Monte in the other years.   The average annual rainfall for the 5 years previous to the study was 11.69 inches, with a variation of 7.21–16.06 inches.   The average annual mean temperature for this period was 55.6°F. with a variation of 24°F.–95°F.

TABLE 1
Physical character of the soil

DEPTH	SIZES OF SOIL PARTICLES				WATER RELATIONS				COMBUSTIBLE MATERIAL
	More than 0.589 mm.	0.588–0.295 mm.	0.295–0.104 mm.	Less than 0.104 mm.	Water content	Water capacity	Optimum water content*	Relative water content†	
inches	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
6	0.718	65.463	33.636	0.099	2.28	24.55	17.19	9.40	0.03
12	0.553	60.182	39.126	0.052	2.89	23.15	16.21	12.48	0.11
24	0.190	54.631	45.188	0.030	2.94	23.37	16.36	12.58	0.09
All	0.493	60.269	39.138	0.058	2.70	23.69	16.58	11.49	0.07

* 70 per cent capacity.

† Percentage of capacity.

TABLE 2
Chemical character of the soil

DEPTH	REACTION	WATER-SOLUBLE SALTS*				
		Total	CO₂	SO₄	Cl	NO₂, etc.
inches	pH	per cent	per cent	per cent	per cent	per cent
All	5.9–6.1	0.00452	0.00142	0.00052	0.00075	0.00183

' Analysis by Miss Miriam Dice, who had made three of the other analyses.

TABLE 3
Organisms per gram of fresh soil

DEPTH	NUMBER OF PLATES		TOTAL COUNT		YEASTS OR BACTERIA		ACTINOMYCETES		FUNGI	
	July	October	July	October	July	October	July	October	July	October
inches					per cent	per cent	per cent	per cent	per cent	per cent
6	10	3	41,640	10,000	48.99	70.00	48.75	30.00	3.26	0.00
12	8	3	135,875	126,700	59.81	57.39	38.73	41.28	1.38	1.34
24	8	3	10,162	37,700	65.06	17.77	33.57	80.37	1.35	1.85
All	26	9	62,559	58,133	57.75	49.54	40.67	49.08	1.57	1.37

ANALYSIS OF SOIL

The sand was passed through a Tyler Standard Screen Scale, of which the sieves with openings as near as possible those previously used were selected. It will be seen from table 1 that the sand was exceedingly uniform in size, over 99 per cent of it being between 0.1 and 0.6 mm. in diameter.

All depths considered, 0.49 per cent of the soil was larger than 0.6 mm., and 99.47 per cent, smaller. If the water content of the second area is assumed to have been approximately the same in July as that of the first area, the soil lost in 3 months without rain 90.79 per cent, 60.55 per cent, and 29.25 per cent of dry weight respectively in the three depths.

The highest number of organisms at 12 inches, the lowest at 6 inches, with 24 inches intermediate agrees with the results for Arizona and Indiana (1,

TABLE 4

Colors of colonies on representative plates and of cultures selected for study

GROUP	TOTAL NUMBER	WHITE	YELLOW	ORANGE	RED	FLUORES-CENT	BROWN
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Plates*......................	3,850	99.14	0.54	0.10	0.00†	0.21	0.00†
Cultures.....................	92	52.17	30.43	5.43	9.78	0.00	2.17

* No colors observed on 24-inch plates.
† No red or brown colonies evident on plates, but cultures developed colors.

TABLE 5

Morphology and physiology of forms selected for study

DEPTH	NUMBER STUDIED	MORPHOLOGY OF FORMS						PHYSIOLOGICAL REACTIONS					
		Coccus	Pleomorphic	Rods		Yeasts	Gram-positive*	Fermenters			Digesters		NO₃ reducers
				Non-spore	Spore			Glucose	Sucrose	Lactose	Gelatin	Casein	
inches		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
6	31	19.35	0.00	70.98	9.67	0.00	67.74†	58.06	45.16	29.03	35.48	29.03	16.12
12	29	6.89	6.89	82.77	3.44	0.00	37.93‡	41.37	34.48	13.79	48.27	34.48	10.34
24	32	9.37	12.50	43.70	28.12	6.25‖	62.50§	56.25**	37.50**	31.25	28.12	37.50	34.37
All	92	11.96	6.52	65.21	14.12	2.17	56.52	52.17	39.13	25.00	36.95	33.69	20.65

* All others were negative.
† All cocci gram-positive and 60 per cent of others.
‡ All cocci gram-positive and 33.33 per cent of others.
§ All cocci gram-positive and 59.25 per cent of others.
‖ One questioned.
** One culture from 24 inches produced gas in glucose and sucrose.

I, III), but only in the case of Arizona do numbers and water content correlate. Massachusetts dunes (I, II) had the highest number at 6 inches and decreased in count downwards, whereas the water content was reversed.

There was gas in glucose and sucrose for one form. This and the large percentage of lactose fermenters are rather unusual, and might indicate contamination by animals except for the fact that there was no gas in lactose. Morphologically the lactose fermenters were divided as follows: 6 inches, 7 short

rods, 1 spore former, and 1 coccus; 12 inches, 3 short rods and 1 spore former; 24 inches, 1 coccus, 1 pleomorphic form, 1 short rod, and 7 spore formers.

On account of the great diversity of reactions it was not possible to arrange the cultures in large groups. The groups that might be formed by those most alike culturally showed great diversity in morphology.

COMPARISON OF THE ATLANTIC AND PACIFIC SOILS

The Pacific sand, a fine-grained, acid soil containing very little soluble salts and almost no combustible material, agrees in general more closely with that from the northern Atlantic than that from the southern. For example, the water relations of the two are very similar. The total count for the Pacific soil lies between those for the two Atlantic soils, but the high percentage of actinomycetes is noticeable. This again (1, IV) can be attributed neither to water content nor to high pH value, although the percentage was slightly higher in October, after the summer's drying. The plates of the Pacific soil showed a much lower percentage of colored forms than did those of either of the Atlantic soils, but the colors of the cultures studied agreed more nearly with those from the northern than the southern Atlantic area.

Morphologically the cultures from the three soils vary rather widely although the percentages of spore formers in the Pacific and northern Atlantic areas agree fairly closely. The much greater fermentative activity of the Pacific soil is a marked difference between the soils compared, particularly in the case of the 25 per cent lactose fermenters and the one culture that formed gas in glucose and sucrose. With the exception of the rather unusual form noted in the North Carolina sand (1, IV) this is the only case of gas formation encountered in the entire study. There is in the Pacific sand noticeably less digestive action but approximately the same amount of reduction as in the northern soil. The southern Atlantic sand differs rather widely on these two points from the other two soils.

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THE AMPHOTERIC NATURE OF THREE COASTAL PLAIN SOILS: I. IN RELATION TO PLANT GROWTH

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In a former publication (4, XII) the relation of the amphoteric nature of certain soil colloids (Sharkey, Sassafras, and Nipe) and the ionization of the basoid group, together with the resultant injury to plant growth was indicated. The soils used in that study were mineral soils from widely separated places representing soils in different categories and characterized by colloids with different silica sesquioxide ratios (3.18, 1.89, 0.31). From the standpoint of aluminum toxicity, mineral soils with colloids of low silica/alumina ratios show toxicity at higher pH values than do soils with wide ratios. In a study of some of the factors that influence the growth of beets, spinach, and other vegetable crops under field conditions within the coastal plain of Virginia, certain relations between soil type, organic matter, pH values, and plant growth were discovered. These crops were found to grow more satisfactorily on some soil types than on others, and in all cases the soils with high organic matter supported crops at lower pH values. The purpose of the experiment herein reported is to note the interrelation between the amphoteric properties of different soil types found locally and the growth of vegetable crops.

The particular soils used in this study were virgin soils[1] and were classified as follows: Portsmouth loamy fine sand (2), Bladen sandy loam (5), and Norfolk very fine sand (2). Briefly, these soils may lie in rather close proximity but by virtue of their position have developed rather definite characteristics. As a result of good surface and internal drainage the Norfolk soils have developed a mature profile comparatively highly weathered. Because of their low organic matter content and comparatively high silica-sesquioxide ratio, they have a relatively high ultimate pH value (4, XII). The Portsmouth soils may have the same parent material in many instances as the Norfolk soils, but by virtue of their low position have not developed a mature profile. They have, however, accumulated a high organic matter content. These two factors give rise to a colloid of a distinctly different nature from the Norfolk—of a more acid character and lower ultimate pH. The Bladen soils generally are derived from beds of heavy clay or heavy fine clay which lie close to tidal

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marsh. These materials have undergone very little aeration and oxidation and in some cases only a slight amount of eluviation in the surface soil which has given rise to a colloid of a rather definite composition. The soil herein used had a relatively low ultimate pH value, was rather low in organic matter, and possessed a colloid of comparatively wide silica/alumina ratio. Table 1 gives comparative data for the three soils.

<center>EXPERIMENTAL PROCEDURE</center>

The soils were brought to the greenhouse and while still moist were placed in 2-gallon earthenware crocks. Large rocks, then fine rocks, and finally silica sand were placed in the bottom of the crocks to insure uniform leaching. The soils were treated with lime or organic matter, as indicated in table 2, before being placed in the crocks. The soil reactions were varied by the use of hydrated lime. The moisture was maintained at an optimum content through-

<center>TABLE 1</center>
<center>*Data concerning soils under study*</center>
<center>(Results Calculated to Soil Dried at 110°C.)</center>

NAME OF SOIL SERIES	BAND*	SILT	CLAY	LOSS ON IGNITION	ORGANIC MATTER	NITROGEN	SOIL pH		REPLACEABLE BASES M.E. 1,000 GM. SOIL			
							Virgin State	Ultimate† State	CaO	MgO	K₂O	Exchange capacity (to pH 7.0)
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*						
Portsmouth..	83	4	5	13.60	7.71	0.319	4.1	3.5	15.3	9.2	2.9	274
Bladen......	75	11	12	3.10	1.80	0.059	4.4	3.6	6.8	6.6	2.0	102
Norfolk.....	90	4	5	1.60	0.93	0.048	5.0	4.7	5.1	5.9	1.4	48

* By the pipette method. Organic matter destroyed by H_2O_2.

† Electrodialyzed soil and water ratio 1:2. pH by quinhydrone and colorimetric methods.

out the growth period. The following data briefly summarized the treatment of the soils:

Beet Crop.—Five beet plants 2 cm. high were set June 13, 1933; fertilized June 26 and July 7; harvested July 25. Each pot received 1.02 gm. of nitrogen, 0.93 gm. P_2O_5, and 0.93 gm. K_2O. The P_2O_5 was from ammophos; K_2O from KCl; N from ammophos, Na NO_3, and $(NH_4)_2$ SO_4. The weight of soil (110°C.) in the pots was as follows: Portsmouth 5,200 gm., Bladen 6,800, and Norfolk 7,700. On 7/17/33 the soils were leached with rain water until a volume of about 1,500 ml. was obtained, or an equivalent of nearly 2 inches of rain. Certain of the results are given in table 2.

Strawberry Crop.—After the removal of the beets, three well-developed strawberry plants (Heflin) were set in each pot on July 27 and harvested on October 30. The crop neither received any further fertilizer treatment nor was the soil leached during or after its growth. The yield data are given in table 2.

TABLE 2

Effect of Soil Acidity and Organic Matter on the Growth of Crops and the Solubility of Aluminum in Different Soil Types

BEETS			STRAWBERRIES		SPINACH		
Dry total weight—roots and tops	Mean pH	Al_2O_3 leached	Dry weight—tops	Mean pH	Dry weight—tops	Mean pH	Al_2O_3 leached
Portsmouth							
gm.		*mgm.*	*gm.*		*gm.*		*mgm.*
0.1	4.0	11.62	1.3	4.0	0.1	4.0	148.96
6.9	4.8	0.22	6.2	4.6	0.6	4.4	1.58
11.6	5.4	None	9.1	5.2	4.5	5.0	0.06
12.0	5.8	None	8.4	5.6	5.2	5.5	None
12.7	6.1	None	10.8	5.7	5.3	5.7	None
13.3	6.2	None	9.6	5.9	6.1	5.8	None
Bladen							
....	4.2	0.86	1.6	4.4	...	4.4	65.35
4.8	4.9	0.06	2.3	4.8	...	4.5	29.00
7.0	5.1	None	4.6	4.9	0.6	4.8	15.70
8.4	6.1	None	6.7	5.6	3.5	5.4	0.03
7.2	6.7	None	3.5	6.3	4.3	6.3	None
Organic matter at low pH (1, 2, 4 per cent)							
1.4		None	4.2				
8.9		None	5.1				
10.8		None	7.1				
Organic matter at optimum pH (1, 2, 4 per cent)							
9.2		None	6.3		3.3		None
10.5		None	6.6		3.5		None
12.7		None	8.3		3.7		None
Norfolk							
0.2	5.5	2.37	....	5.2	...	4.8	302.68
3.5	5.7	None	1.1	5.4	...	5.0	3.33
4.5	6.2	None	2.4	5.6	3.9	5.5	0.26
3.7	6.6	None	7.2	5.8	6.7	6.0	None
3.0	7.0	None	6.7	6.2	5.6	6.2	None
Organic matter at low pH (1, 2, 4 per cent)							
6.6		None	2.3		0.8		11.52
9.1		None	6.0		1.6		8.06
11.9		None	11.9		2.4		1.33
Organic matter at optimum pH (1, 2, 4 per cent)							
9.5		None	6.0		5.0		None
12.7		None	10.5		6.7		None
12.8		None	11.6		5.8		None

Spinach Crop.—On November 13 the soil in each pot was planted to 10 hills of spinach (Virginia Savoy). The seeds were treated with red oxide of copper to prevent damping off. On November 25 each pot was fertilized with one-half the quantity of fertilizer from the same source as that used on the beets. The pots were leached a few days before harvest until about 1,500 ml. of water was collected. The yield data and aluminum leached are also shown in table 2.

Soil reaction and plant growth

The soil colloid constitutes complexes of weak acids (silicic, humic, etc.) and bases (aluminum, iron, etc.) which only partially neutralize each other, leaving free valences for the absorption of bases at high pH values (4, X). The extent of the excess of acids over the bases and the quantity of the material in the soil control the absorbing power of a soil for lime. The Portsmouth soil has a base holding capacity at pH 7, nearly three times that of the Bladen, principally because of the higher organic content (1, 8). The ultimate reaction (4, X) of the soils was pH 3.5 for the Portsmouth and pH 3.6 for the Bladen, whereas that for the Norfolk soil was pH 4.7. The differences in the composition of these soil colloids were reflected in the point of exchange neutrality (the pH of the electrodialyzed soil in a N Na_2SO_4 solution), which was Portsmouth 3.8, Bladen 4.2, and Norfolk 5.0. The lowest pH value that these soils can show without the disintegration of the soil colloid is reflected by this point. This in turn indirectly effects the lowest pH at which plants will make good growth. These readings are about as indicative of the character of the soil colloids as is a chemical analysis. They reflect that the acidoid/basoid ratio widens progressively as follows: Norfolk, Bladen, Portsmouth. The organic or humus content of the Portsmouth is principally responsible for its high acidoid content, which would not necessarily be reflected in the silica-sesquioxide ratio.

For the beet crop the lowest reaction for good growth in the Portsmouth was pH 4.8, or about 25 per cent saturated in respect to calcium; pH 4.9, or 20 per cent saturated, for the Bladen; pH 5.7, or 18 per cent saturated, for the Norfolk soil. Very marked increases in yields were obtained at higher reactions, however. A reaction of pH 6.0 or slightly above (9) seems to be very favorable for the growth of beets in these soils.

The addition of organic matter to the Bladen and Norfolk soil lowered the reaction at which beets grew satisfactorily. The source of organic matter was peat moss which was exceedingly low in available plant nutrients and had a low pH value. The peat moss carried a pH of 3.8, 0.707 per cent nitrogen, and 5.43 per cent ash content. The benefit of the peat moss, then, was not in the nutrients that it carried but in the physico-chemical action on the soil colloid; that is, it increased the active (1, 3, 8) acidoid constituent. The fact that it did not greatly increase the yields at the optimum reaction in the Bladen, but did in the Norfolk soil, further supports this view.

For the strawberry crop the lowest reactions for good growth were pH 4.6, 4.9, and 5.8 for the Portsmouth, Bladen, and Norfolk soils respectively. Although the strawberry plant is often considered to be very tolerant of an acid soil, these results indicate that it is about as sensitive to an unfavorable soil condition as are beets. Probably the conception that the strawberry plant will grow at low pH values has arisen from the fact that strawberries are usually grown on low-lying soils that carry a high organic content. The response to organic matter in the Bladen and Norfolk soils further supports this view.

The grower has found that spinach, possibly more than most of the vegetable crops, has responded to liming practices. The results shown in table 2 indicate at least that this crop is very responsive to a change in soil reaction toward the neutral pH. The lowest pH readings for good growth were 5.0, 5.4, and between 5.5 and 6.0 for the Portsmouth, Bladen, and Norfolk soils. Spinach showed also the characteristic response to increased organic matter content of the soils.

Soluble aluminum

The significance of the effect of soluble aluminum in acid soils upon plant growth has been pointed out by many workers (4, XII; 6). Attention has also been called to the fact that aluminum and other components of the basoid group ionize and become soluble at different pH values in soil types with different isoelectric points. For example, a soil with a high ultimate pH represents one with a relatively active basoid group in which the aluminum is ionizable and soluble at a comparative high pH value and *vice versa* for a soil with a low ultimate pH value. Further indications of these facts are noted in the pH of exchange neutrality. As shown in table 2, aluminum appeared in solution at pH 4.8 in the Portsmouth soil, at pH 4.9 in the Bladen, and in comparatively large quantities at pH 5.5 in the Norfolk soil during the growth of the beet crop and at a slightly higher pH for the spinach crop. At a pH of 5.5 neither the Bladen nor the Portsmouth soil showed any aluminum in the drainage water during the growth of beets. Further, plant growth was very good at this reaction in these two soils, which is all in agreement with the amphoteric nature of the soil. A somewhat similar relation was observed between these points for the Sharkey, Sassafras, and Nipe soils (4, XII).

The addition of a strongly acidoid material to a soil of the Norfolk type increases the acidic properties, thereby suppressing the activity of the basoid group. This action suppresses the ionization and solubility of the basoid group, which in turn suppresses the fixing power of the soil for phosphorus. Data on the leaching of phosphorus from the soil is given in part II of this paper.

The data in table 2 show that much larger quantities of aluminum were leached from the soil at low pH values after the growing of the spinach than after the beet crop. Several factors account for this large difference. The lowering of the soil reaction from the effects of acid-forming fertilizers and of

leaching certainly influenced the leaching of aluminum. A small lowering of the soil reaction at the critical point makes a big difference in the leaching of the basoid constituent. Further, less phosphorus was added during the growth of the spinach than in the growing of the beets. Phosphorus was practically negative in the leaching water from the spinach, whereas it was much higher in the drainage water from the beets. Many experimental data (6, 9) show that through the addition of heavy applications of phosphorus to acid soils plants grow better and that this growth is due to the removal of aluminum from solution. Since the Norfolk soil has a strong power for fixing phosphorus (*see* part II), the growth of the crops at the optimum pH was not so good as on the other two soils. When organic matter was added to the Norfolk soil, thereby lessening the power to fix phosphorus, the crops grew as well as on the other soils. Further work in progress shows clearly that low availability of phosphorus was responsible for the depressed yields in the Norfolk soil. Part II deals with the leaching and absorption of soil constituents on the different soil types.

SUMMARY

Three soils found in the coastal plain of Virginia, a Portsmouth loamy fine sand, a Bladen sandy loam, and a Norfolk very fine sand, with ultimate pH values of 3.5, 3.6, and 4.7, respectively, were selected for a study of the effect of soil acidity upon the growth of vegetables. The lowest reactions for satisfactory growth of beets on the Portsmouth series was pH 4.8; on the Bladen series, 4.9; and on the Norfolk, 5.7, or at approximately the same percentage of calcium saturation. For strawberries the lowest reaction for good growth was pH 4.6, 4.9, and 5.8; for spinach 5.0, 5.4, and between 5.5 and 6.0, respectively, for the Portsmouth, Bladen, and Norfolk soils. The point at which growth was markedly retarded was directly correlated with the appearance of aluminum in the drainage water. The addition of organic matter, in the form of peat moss, to the Bladen and Norfolk soils suppressed aluminum solubility at low pH values and enabled crops to grow more satisfactorily than in the untreated soils.

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PLATE 1

EFFECT OF SOIL TYPE, REACTION, AND ORGANIC MATTER UPON THE GROWTH
OF BEETS

FIG. 1. Effect of soil type and reaction.
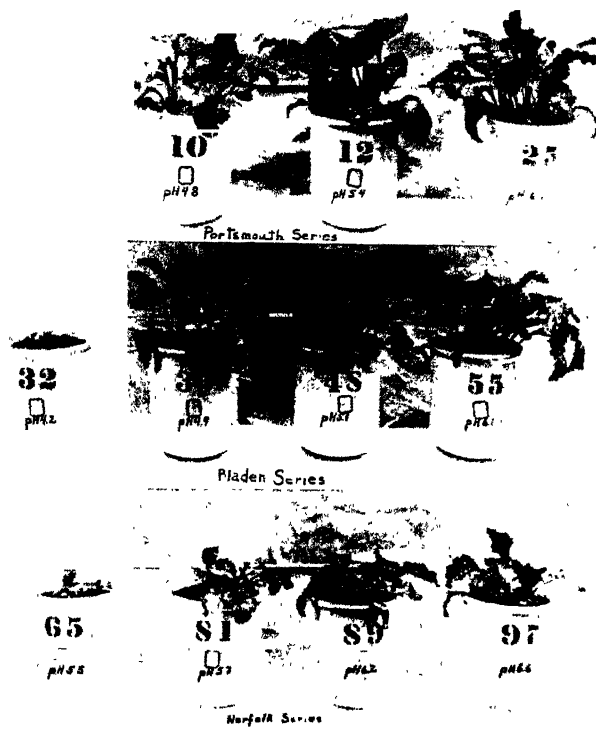FIG. 2. Effect of soil type and organic matter (peat moss source of organic matter).

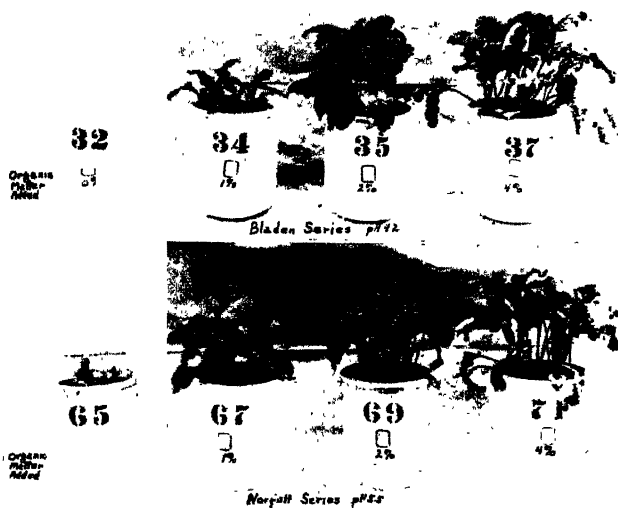JACKSON B. HESTER

FIG. 1

FIG. 2

THE AMPHOTERIC NATURE OF THREE COASTAL PLAIN SOILS: II. IN RELATION TO THE LEACHING AND ABSORPTION OF SOIL CONSTITUENTS BY PLANTS

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ANALYTICAL DATA

As explained in Part I, a crop of beets was grown upon the soil until it was evident that the maximum growth had been reached. The soil was then leached with approximately 1,500 ml. of rain water, the equivalent of almost 2 inches of rain. The chemical analyses[1] of the drainage water and plant material for certain nutrients are given in table 3. The first row of figures in each soil series mentioned represents the virgin soil with no treatment other than fertilization. Table 4 gives the percentage of the replaceable bases removed by plant absorption and leaching. The assumption that the potash applied was replaceable is possibly incorrect. The Bladen soil apparently has a high fixing power for potash. Table 5 gives the percentage of total nitrogen and the percentage of the added phosphorus leached and absorbed by the plant. The soils were leached while the spinach was growing and the analytical results are given in table 6. The data in tables 3–6 are given to show the behavior of the three soil types under these conditions and are discussed from that point of view.

Calcium

The quantity of calcium leached increased with a rise in pH but not in proportion to the quantity of lime added. For example, as expressed in table 4, 15 per cent of the replaceable lime in the Portsmouth soil was leached at pH 4.0, whereas only 2 per cent was leached at pH 6.2. A similar relation held true for the Bladen and Norfolk soils. There was an increase in absorption by the plants with a rise in pH until the optimum growth was attained, or in other words, the higher the crop yield the greater the absorption of calcium. The total quantity of replaceable calcium was in the order Portsmouth, Bladen, Norfolk. The leaching of the lime maintained the same order, but the percentage of the total replaceable leached was in the reverse order. In other words, the soil that had the greatest exchange capacity had the strongest combining power for lime. Since the Norfolk soil has a low base exchange capacity

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[1] Florence A. Shelton assisted in making the chemical analyses.

the removal of calcium (on the percentage of total replaceable) was the highest of the three soils at any given pH value.

Magnesium

The results of the leaching and absorption of magnesium indicate that plant absorption is a big factor in the removal of magnesium from the soil. Since no magnesium was added, all removed had to be accounted for by that present

TABLE 3

Effect of soil type and soil reaction upon the leaching and absorption of nutrients by plants

(After beet crop)

| MEAN pH | M.E. PER 1,000 GM. OF SOIL (110° C.) | | | | | | | | | |
| | Calcium | | Magnesium | | Potassium | | Phosphorus | | Nitrogen | |
	Leached	Absorbed	Leached	Absorbed	Leached	Absorbed	Leached	Absorbed	Leached	Absorbed
Portsmouth										
4.0	2.38	....	1.09	....	1.73	....	0.01	....	6.78	....
4.8	3.88	0.91	0.60	0.46	0.66	1.07	0.11	0.34	5.20	4.19
5.4	3.41	1.34	0.45	1.09	0.69	2.30	0.29	0.46	3.71	6.24
5.8	3.72	1.43	0.35	1.00	0.41	2.75	0.23	0.47	3.69	6.75
6.1	4.33	1.57	0.34	1.22	0.34	3.35	0.18	0.47	4.41	6.53
6.2	6.15	1.71	0.40	1.20	0.34	3.68	0.13	0.53	6.31	7.30
Bladen										
4.2	1.31	....	0.56	....	0.38	....	0.03	....	2.48	....
4.9	1.12	0.27	0.29	0.39	0.21	0.43	0.03	0.14	1.12	2.13
5.1	1.20	0.49	0.21	0.51	0.26	0.70	0.03	0.21	1.64	3.27
6.1	2.28	0.64	0.26	0.49	0.12	1.00	0.06	0.25	1.04	3.80
6.7	2.41	0.63	0.19	0.32	0.13	0.95	0.07	0.21	1.89	3.45
Norfolk										
5.5	1.13	....	0.53	....	1.06	....	0.005	0.01	4.31	....
5.7	1.92	0.23	0.37	0.15	0.81	0.33	0.01	0.09	1.91	1.54
6.2	2.49	0.27	0.34	0.19	0.52	0.75	0.01	0.11	2.92	1.91
6.6	4.37	0.19	0.32	0.21	1.10	0.58	0.015	0.09	4.49	1.55
7.0	3.99	0.15	0.31	0.17	0.88	0.47	0.025	0.07	4.65	1.21

in the soil. The leaching of magnesium at low pH values was very high in proportion to the total removal at high pH values. The fact that a great deal of the available magnesium is removed from the soil by leaching has been shown in the Norfolk area in connection with magnesium deficiency on potatoes. During rainy years this deficiency becomes apparent over the entire trucking area, but is observed to a much more limited extent in dry years (2). The analyses of the drainage water and plant material were, therefore, a good indication of the relative available magnesium under these conditions.

Potassium

The Bladen soil possesses a high power for fixing potash in a non-replaceable state. Many soils apparently (8, 11, 12, 14, 16) show this property. The total removed from this soil is low in comparison to the other two soils. The removal from the Portsmouth by leaching is fairly low, but plant absorption is high, the total utilization by one crop of beets being 85.2 per cent of the replaceable and added potassium at the optimum reaction. The total removed

TABLE 4

Effect of soil type and soil reaction upon the leaching and absorption of replaceable bases

(After beet crop)

MEAN pH	PER CENT OF REPLACEABLE BASES* IN SOIL REMOVED BY LEACHING AND PLANT ABSORPTION								
	Calcium			Magnesium			Potassium		
	Leached	Absorbed	Total	Leached	Absorbed	Total	Leached	Absorbed	Total
Portsmouth									
4.0	15.55	0.00	15.55	11.85	0.00	11.85	36.7	0.0	36.7
4.7	4.44	1.04	5.48	6.53	5.00	11.53	14.0	22.7	36.7
5.4	2.11	0.82	2.93	4.90	11.85	16.75	14.6	48.8	63.4
5.8	1.79	0.69	2.48	3.81	10.76	14.57	8.7	58.3	67.0
6.1	1.69	0.61	2.30	3.70	13.27	16.97	7.2	71.0	78.2
6.2	2.03	0.56	2.69	4.35	13.05	17.40	7.2	78.0	85.2
Bladen									
4.2	18.67	0.00	18.67	8.49	0.00	8.49	11.2	0.0	11.2
4.9	3.86	0.97	4.83	4.40	5.91	10.31	6.2	12.7	18.9
5.1	2.34	0.96	3.30	3.18	7.73	10.91	7.7	20.7	28.4
6.1	3.11	0.87	3.98	3.94	7.43	11.37	3.5	29.5	33.0
6.7	1.72	0.45	2.17	2.88	4.70	7.58	3.8	28.0	41.8
Norfolk									
5.5	22.05	0.00	22.05	9.00	0.00	9.00	42.6	0.0	42.6
5.7	12.99	1.55	14.53	6.10	2.54	8.64	31.2	13.3	44.5
6.2	10.21	1.10	11.31	5.76	3.22	8.98	19.8	28.5	48.3
6.6	9.95	0.46	10.41	5.42	3.56	8.89	41.5	22.0	63.5
7.0	4.84	0.19	5.03	5.26	2.88	8.14	33.1	17.9	51.0

* Assuming that added nutrients were in replaceable state.

in the Norfolk soil was high, with leaching accounting for the greater proportion removed. This method of studying the relative available potash for the crops used was exceptionally well adapted to these soils.

Phosphorus

The Portsmouth, Bladen, and Norfolk soils received an application of 7.7, 5.9, and 5.2 m.e. of phosphorus per 1,000 gm. of soil for the beet crop. The

data in table 3 show that only a small proportion of this was removed either through leaching or by the plants. The absorption by the plants and the leaching were influenced markedly by the soil reaction, organic matter content, and amphoteric point of the soil. The amount of phosphorus removed from the soil by leaching following the growth of spinach was so small that it is not mentioned except in the Portsmouth soil.

A great deal has been said (3, 5, 6, 14, 17) about the fixation of phosphates in different soils. Sundry compounds (4, 6, 12, 13) of different natures have

TABLE 5

Effect of soil type and soil reaction upon the leaching and absorption of nitrogen and phosphorus
(After beet crop)

PER CENT TOTAL NITROGEN IN SOIL REMOVED			PER CENT OF ADDED PHOSPHORUS REMOVED		
By leaching	By plant	Total	By leaching	By plant	Total
Portsmouth					
2.72	0.00	2.72	0.08	0.00	0.08
2.10	1.68	3.78	0.87	2.70	3.57
1.49	2.51	4.00	2.30	3.65	5.95
1.48	2.71	4.19	1.83	3.73	5.56
1.76	2.62	4.38	1.43	3.73	5.16
2.53	2.93	5.46	1.03	4.21	5.24
Bladen					
4.63	0.00	4.63	0.31	0.00	0.31
2.10	3.98	6.08	0.31	1.46	1.77
3.05	6.10	9.15	0.31	2.20	2.51
1.93	7.07	9.00	0.62	2.60	3.22
3.52	6.40	9.92	0.73	2.20	2.93
Norfolk					
9.92	0.00	9.92	0.02	0.12	0.14
4.42	3.55	7.97	0.12	0.95	1.07
6.68	10.05	16.73	0.12	1.17	1.29
10.30	13.85	24.15	0.18	0.95	1.13
10.65	13.54	24.19	0.26	0.82	1.08

been shown to account for this absorption. Since clays show amphoteric properties (1; 7; 11, VI) the question of the fixation of phosphates in acid soils is indicated. For example, soils with a high amphoteric point (laterites, etc.) fix larger amounts of phosphates than do soils with low amphoteric points (7; 11, VI). Because of the amphoteric nature of soils, more phosphorus is fixed at low pH values than at high reactions. Soils with low organic content fix greater quantities of phosphorus than do similar soils with high organic content. The addition of electronegative material, silicates and humates, reduced the quantity of anion fixed by the colloid.

We find only a low utilization of phosphorus in all of these soils.   However, the results in table 4 and the calculations in table 5 indicate that the soils have fixing powers for phosphorus which may be expressed in the order Norfolk, Bladen, Portsmouth.   The utilization by the plants and the leaching of phosphorus were in the reverse order of fixation.   The additions of a strongly electronegative material (humus) which satisfied some of the positive valences of the colloidal complex prevented somewhat the fixation of the phosphorus, thus permitting its better utilization by the plants.   The low availability of

TABLE 6

Effect of soil type and soil reaction upon the leaching and absorption of nutrients by plants
(After spinach crop)

MEAN pH	M.E. PER 1,000 GM. OF SOIL (110°C.)									
	Calcium		Magnesium		Potassium		Phosphorus		Nitrogen	
	Leached	Absorbed	Leached	Absorbed	Leached	Absorbed	Leached	Absorbed	Leached	Absorbed
Portsmouth										
4.0	2.18	....	1.24	....	0.97	....	0.002	....	6.75	....
4.4	7.36	....	1.30	....	0.68	....	0.006	....	7.45	....
5.0	7.07	0.86	0.80	0.54	0.21	1.42	0.008	0.31	4.84	2.88
5.5	6.54	1.18	0.57	0.68	0.14	1.73	0.05	0.39	4.10	3.60
5.7	6.65	1.22	0.51	0.69	0.09	1.80	0.061	0.40	4.40	3.10
5.8	7.90	1.41	0.71	0.85	0.10	2.11	0.062	0.41	4.74	3.98
Bladen										
4.4	2.35	....	0.94	....	0.71	....	.....	....	4.88	....
4.5	3.97	....	0.78	....	0.41	....	.....	....	4.67	....
4.8	4.13	0.09	0.56	0.03	0.14	0.06	.....	0.03	3.84	0.19
5.4	4.17	0.46	0.32	0.32	0.06	0.62	.....	0.16	2.91	1.46
6.3	5.70	0.67	0.48	0.32	0.09	0.95	.....	0.27	3.06	1.63
Norfolk										
4.8	1.65	....	0.64	....	1.23	....	.....	....	4.12	....
5.0	4.15	.:..	0.86	....	0.81	....	.....	....	5.35	....
5.5	4.97	0.26	0.80	0.22	0.41	0.50	.....	0.11	4.77	1.06
6.0	5.28	0.50	0.62	0.46	0.22	1.55	.....	0.28	3.54	2.66
6.2	6.77	0.45	0.69	0.34	0.15	1.28	.....	0.21	2.99	2.16

phosphorus in the Norfolk soil was no doubt responsible for low crop yield. Then the addition of organic matter overcame this deficiency by preventing to some extent the fixation of the phosphorus, and thereby brought up the crop yield to the maximum for any of the three soils.   A very high fixation of phosphates at low pH values was noted in all of these soils.   The estimation of the phosphorus by chemical analyses of the leaching water and plant material was very satisfactory under these conditions for determining its relative availability.

Nitrogen

The carbon/nitrogen ratio of the three soils used—Portsmouth, Bladen, and Norfolk—was 14, 17, and 11 respectively. From this standpoint (15, 16) the availability of nitrogen would be the least in the Bladen and the highest in the Portsmouth and Norfolk soils. The largest amount of removed nitrogen, found in the Portsmouth soil, was due to the high state of fertility of the soil. The addition of lime greatly stimulated the bacterial activity (16) and likewise the nitrogen released from the organic matter of the soil. Since there were added 14, 11, and 10 m.e. of nitrogen per 1,000 gm. of soil for the Portsmouth, Bladen, and Norfolk, the removal was almost 100 per cent for the Portsmouth, about 50 per cent for the Bladen, and more than 60 per cent for the Norfolk soil, at relatively high pH values during the growth of the beet crop. The addition of organic matter stimulated high utilization of nitrogen on the Norfolk and Bladen soils, not because of the extra added nitrogen, but because of its physico-chemical action on the soil.

DISCUSSION

The colloidal complexes represented in these three soils differ both in quality and quantity as indicated by their amphoteric points and exchange capacity. These differences manifest themselves in various ways: First, in the quantity of lime necessary to neutralize the acidity of the soil. The CaO holding capacity of the soils in pounds per acre, 0–6$\frac{2}{3}$ inch basis, at pH 7.0 on the basis of their specific gravity was 11,850, 5,720, and 2,760 for the Portsmouth, Bladen, and Norfolk soils respectively. This difference is accounted for quantitatively in the amount of colloid in the soil and qualitatively by the difference in the composition of the colloid. Although the Portsmouth soil carried more replaceably lime, the leaching of the lime from the soil was, in terms of the percentage of the total replaceable, less than that from the Bladen, which was in turn less than that of the Norfolk soil. This is accounted for by the fact that the Portsmouth soil carried a colloid with relatively strong acidic properties, low ultimate pH, and low isoelectric point. Secondly, the Bladen soil had a low ultimate pH but less active colloid than did the Portsmouth soil. The Norfolk soil had a relatively high ultimate pH and a smaller quantity of colloid to a given amount of soil. These two factors, then, account for its low exchange capacity. Although all of the soils have a relatively strong power for fixing phosphorus, the Norfolk soil showed the greatest power. At low pH values all three soils showed very strong phosphate fixing powers.

Since the soils show differences in their power to give up cations (9; 11, XI) and in their power to fix phosphates (7, 11, 16, 15, 20), and further, since crops show different nutrient requirements (15) and different powers to absorb (15, 18) them, and since these differences are shown by the analyses of the leaching water and plant material, the author believes that some form of the aforementioned method will be valuable in determining the relative availability of plant nutrients and toxants for a specific crop.

Leaching of Organic Matter

As a result of the favorable reaction in the soil brought about by the application of lime, the bacterial activity of the soil was greatly increased (21) as the reaction approached neutrality. This affected the soil processes in several ways, such as the liberation of plant nutrients and other soil constituents. As the soil reaction in each soil type approached the neutral pH reading, the amount of organic matter in the leaching water definitely increased. These results were somewhat more pronounced in the first leaching than in the second. These data are given in table 7. The leaching of the organic matter is associated with the amphoteric points of the soils. For example, there is about as much organic matter leached at pH 6.2 in the Norfolk soil as at pH 6.1 in the Portsmouth soil even though the Portsmouth soil contains about seven times as much organic matter. Again, at the reaction pH 5.5 in the Norfolk soil comparatively little organic matter is leached, whereas a reaction of pH 4.0 was reached before the Portsmouth soil showed as low leaching of organic matter. The Bladen soil showed little or no organic matter leached at pH 4.2.

TABLE 7

Influence of soil reaction upon the leaching of organic matter

PORTSMOUTH		BLADEN		NORFOLK	
Mean pH	Organic matter leached	Mean pH	Organic matter leached	Mean pH	Organic matter leached
	mgm.		*mgm.*		*mgm.*
4.0	5.85	4.2	0.00	5.5	6.66
4.8	13.14	4.9	2.02	5.7	22.90
5.4	29.82	5.1	4.04	6.2	27.30
5.8	23.61	6.1	6.00	6.6	81.10
6.2	41.80	6.7	10.20	7.0	112.00

SUMMARY

Three soils found in the coastal plains of Virginia represent soils with wide variations in cation exchange capacity, replaceable bases, pH value, and organic matter content. These soils show different amphoteric points, which reflect differences in the leaching and absorption of soil constituents.

Data are given to show the advantage of using the analysis of the plants and the drainage water from pot cultures to show the limiting element in plant growth and the available nutrients and toxants for specific crops in these soils.

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Keijiro Aso

KEIJIRO ASO

To Be Honored by Japanese Agricultural Societies

The sixtieth birthday of Keijiro Aso and his 35 years of continuous service on the faculty of the Tokyo Imperial University will be celebrated on April 7 in Tokyo at the annual meeting of the scientific agricultural societies of Japan.

Doctor Aso was born in Tokyo in 1875. He was graduated from the course in agricultural chemistry at Tokyo University in 1899 and received the degree of doctor of agriculture (Nogaku-Hakushi) upon completion of the University Hall (the post-graduate course) in 1904. In 1901, he was appointed Assistant Professor and sent by the government to study for three years the science of soils and manures in Europe and in America. He studied for one year under Professors Raman and Hiltner in München, and for two years under Professors Lemmermann, Wahnsshaffe, and Nerst in Berlin. He received instruction also from Professor Treitz in Budapest.

He attended the Second International Conference of Soil Science at Stockholm in 1910, and also served on one of the exhibition juries at the world exposition held at Turin, Italy, in 1911.

On his return to Tokyo, Doctor Aso was appointed Professor of Agricultural Chemistry and also served as an inspector of the Department of Education. Meantime he was elected President of the Chemical Society of Japan. He founded the Society of the Science of Soil and Manure of Japan and at present is president of that society as well as representative of the Japanese Section of the International Society of Soil Science.

In 1930, Professor Aso was again sent abroad by the government and attended the Second International Congress of Soil Science at Moscow and Leningrad. He was elected one of the General Committee of the International Society of Soil Science at Washington and also at Moscow. After the Congress, he visited Germany, Sweden, Denmark, France, England, and America.

Again returning to Japan, he was elected Dean of the Agricultural Faculty of Tokyo Imperial University. On his sixtieth birthday, in June, he will retire from active service at the University.

A NEW INSTRUMENT FOR SOIL SAMPLING

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In taking samples of soils for the determination of specific gravity and for subsequent chemical analysis, it is important that the samples should be taken vertically from the surface to a prescribed depth or from one prescribed level in the profile of the soil to another, so that the volume of the sample is exactly known. This in order to have a definite basis for an exact determination of the specific gravity and for the calculation of the content of plant nutrients per unit area of the soil, as a rule to a depth of 20 cm.

In order as far as possible to fulfill the demand for exact sampling and for a definite determination of specific gravity, the writer has constructed a special instrument for taking samples of soils predominantly organic in nature and another for mineral soils.[1]

The sampler for organic soils consists of a brass cylinder with an internal diameter of 7.98 cm., thickness of metal 2.0 mm., and height from the cutting edge to the bottom of the cylinder 20.0 cm. (fig. 1 and plate 1, no. 1). The internal volume of the cylinder is consequently exactly 1 liter, and the sample is taken to exactly the required depth, viz., 20 cm. The upper edge of the sampler has a flange 1.5 cm. in height, with an internal diameter of 8.20 cm., into which is fitted a loose lid which forms the bottom of the cylinder (figs. 1, 3 and plate 1, no. 2). The lid is provided with a fixed handle which is large enough to admit one hand. By the aid of four rivets (fig. 1 and plate 1, no. 4) which fit into four slots in the outer edge of the cylinder (fig. 1 and plate 1, no. 5), the lid is easily fastened to the cylinder. The fixed handle can be provided with a lever (fig. 3 and plate 1, no. 7) large enough for both hands if it is necessary to use more force to press the cylinder down into the soil. The loose arm consists of a U-shaped iron plate which covers the fixed handle and, in addition, is fixed by the aid of two rivets (fig. 3 and plate 1, no. 9) which fit into holes with corresponding slots in the fixed handle (fig. 3 and plate 1, no. 8). The sample cylinder is emptied by removing the lid and inserting into the cylinder a perfectly fitting, light, hollow copper cylinder (fig. 2 and plate 1, no. 6), which is provided with a bottom at one end and a handle at the other. The soil is emptied into a box, or the like, which is used for packing. In order to avoid loss of soil or moisture, if a packing-case is not at hand, a lid (fig. 1,

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[1] The soil sampler can be supplied by the Central Scientific Company, Chicago, Illinois.
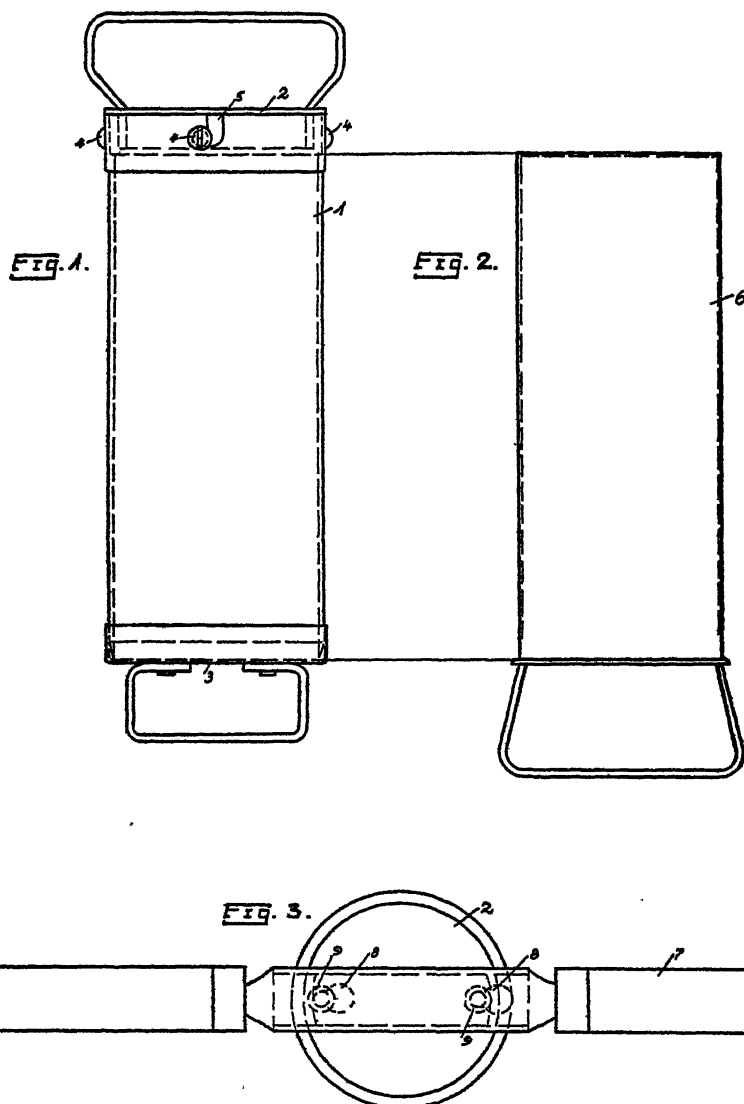
FIGS. 1, 2, AND 3. DIAGRAMS SHOWING THE CONSTRUCTION OF THE SOIL SAMPLER

1—Brass cylinder; *2*—lid forming bottom of cylinder; *3*—lid covering cutting edge of cylinder; *4*—rivets in cover; *5*—slots in cylinder; *6*—inner copper cylinder; *7*—lever upon which additional force can be applied to press cylinder into soil; *8*—slots in fixed handle; *9*—rivets holding loose arm to fixed handle.

no. 3) is fitted on the mouth or cutting edge of the sampler, which to a length of about 7 mm. is cone-shaped.

The sampler and its fittings are packed into a light wooden box with internal dimensions of 34 by 11 by 10 cm. and provided with a handle for easy transportation. The hollow cylinder which is used to empty the sampler is then placed inside the latter.

Solid, drawn brass tubing, a material which after 1 year's testing has proved to be very serviceable for taking samples of organic soil, is recommended for the sampler.

For hard and stony soils the sampler must be smaller and made of a harder material. The writer has therefore constructed for these soils a special sampler of rustless steel, with an internal diameter of 3.57 cm. At a depth of 20 cm., therefore, the volume of the sample is 1/5 liter. In other respects the sampler is exactly like that previously described for soils predominantly organic in nature but in emptying the cylinder, when the soil is very dry, a little T-shaped iron rod with a spade-shaped extension at the end about 5 cm. long and 1.5 cm. wide has been found practical for loosening the soil in the cylinder.

Samples are taken by making a hole in the ground with a spade and removing the vegetation so that the surface is quite level along one edge of the hole. The sample cylinder with lid adjusted and, if desired, with the lever on, is then screwed into the soil to the 20-cm. mark (which, of course, is at the same height as the bottom inside the cylinder) at a suitable distance from the edge of the hole. The spade is then inserted into the wall of the soil just beneath the cylinder, which is then taken out and emptied in the manner already described. If the soil is very loose, the spade can be inserted in the wall of the soil 20 cm. below the surface *before* the sampler is screwed down. If samples of soils are to be taken from deeper layers, suitable soil ledges or steps are made. If samples of thinner strata than 20 cm. are desired, the spade is merely placed in the wall of the soil at the desired distance below the surface or below the top of the soil ledge, and the volume of the sample can be computed from the base and the depth of insertion of the cylinder.

For packing 1.25 liter[2] tin boxes with a spring catch, or rubber bags are recommended. The latter, being quite water-proof, prevent loss of either soil or water during transportation to the laboratory. If samples of soils larger than 1 liter are needed from the same place, in the case of soils predominantly organic in nature, several 1-liter samples can be taken, of course, and either larger boxes used for packing or the samples sent separately and the mixture made in the laboratory. In the case of mineral soils, five samples are taken and mixed at once. The determination of the specific gravity is then a very simple matter, for after the soil has been weighed and its water content determined we have the dry weight of the soil just as it is deposited in nature. It is obvious that this is of importance in the subsequent treatment of the analysis data obtained.

[2] One liter and a quart.

PLATE 1

THE COMPLETE SOIL SAMPLER

For explanation of numbered parts, see figures 1, 2, and 3
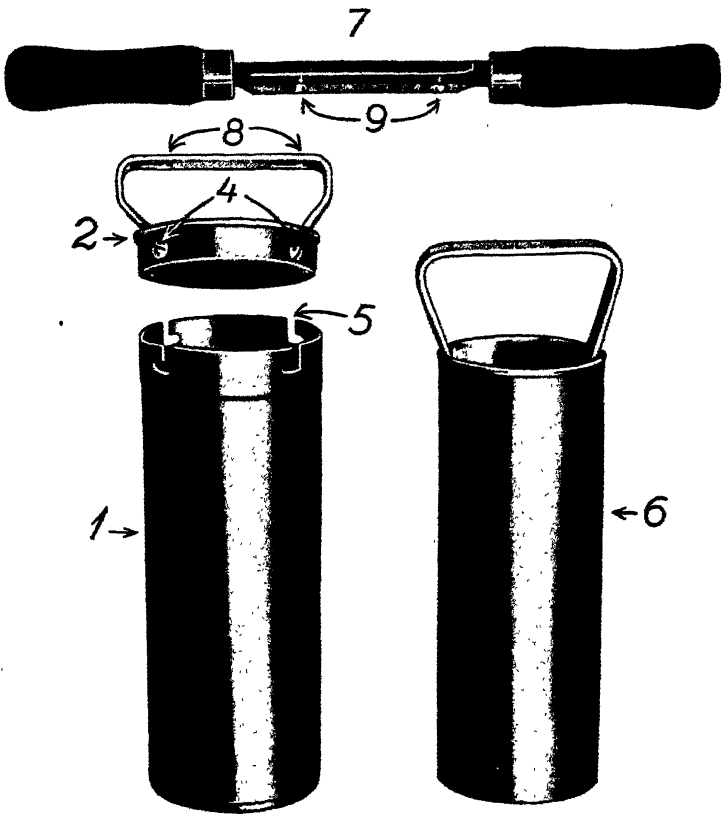
THE AMMONIUM CARBONATE METHOD OF DISPERSING SOILS FOR MECHANICAL ANALYSIS

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During recent years a good deal of attention has been directed toward the methods of dispersing soils for mechanical analysis. The newer methods aim at attaining maximum dispersion so that all the compound particles are broken up into their ultimate units. Working on the original suggestion of Robinson, the International Society of Soil Science and the Agricultural Education Association (England) have adopted the use of 6 per cent hydrogen peroxide for the oxidation of organic matter, though in routine work no distinction is made between soils that require hydrogen peroxide treatment and those that do not, the hydrogen peroxide treatment being generally given to all types of soils. The soil after the hydrogen peroxide treatment is treated with dilute HCl, followed by shaking with water to which 4 cc. of N NaOH has been added.

In the Bureau of Chemistry and Soils method (4) the acid treatment is optional and the dispersing agent is sodium oxalate. In the other three methods; namely, the NaCl method (5), the Sudan method (2), and Troell's method (7) neither H_2O_2 nor HCl is used. In the Sudan method the dispersing agent is Na_2CO_3. This method is not suitable for pipette technique. In the NaCl method proposed by the author, the soil is treated with N NaCl, and after the excess has been removed with a little water, sufficient NaOH is added to make the suspension alkaline to phenolphthalein. In Troell's method the oxidizing agent is sodium hypobromite, after the removal of which the soil is treated with NaCl.

The author has shown elsewhere (5) that the most effective method of dispersing soils is to introduce Na in the exchange complex, and the NaCl method is based on that principle. It was found, however, that certain ferruginous and humus soils did not respond to this treatment, and modification of the method for those types of soils was considered necessary.

In a study on the use of ammonium carbonate as a reagent for determining exchangeable bases in soils (6) the author found that when a soil containing exchangeable Ca is treated with ammonium carbonate, the whole of the replaceable Ca is converted into $CaCO_3$, and ammonium ions take their place. This

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gave the clue to a very simple method of converting calcium soils into sodium soils. The soil is boiled with ammonium carbonate solution followed by heating with NaOH solution, when ammonia is driven off and NH$_3$ ions are replaced by Na in the exchange complex. The application of this technique for the mechanical analysis of soils followed as a matter of course. The use of ammonium carbonate not only proved effective for soils that responded to the NaCl treatment, but it produced maximum dispersion in ferruginous and humus soils for which the NaCl method had not proved satisfactory.

DETAILED DESCRIPTION OF THE AMMONIUM CARBONATE METHOD

From 10 to 20 gm. of soil is boiled on a rose burner with 250 cc. of N (NH$_4$)$_2$CO$_3$ (pure) until the volume is reduced to half. Tall beakers of 600 cc. capacity are found most satisfactory and are permanently marked at 125 and 250 cc. The beaker should be covered with a watch glass for the first 10 minutes or so when the boiling is more vigorous. If the suspension shows a tendency to froth, 10 cc. of kerosene oil or of a mixture of equal parts of kerosene oil and gasoline is added when frothing immediately subsides. When the volume of the suspension has been reduced to about one half, 4 to 8 cc. of N NaOH or LiOH for every 10 gm. of soil is added and the suspension brought back approximately to the original volume by the addition of hot water. The boiling is continued until the volume is again reduced to one half. The suspension needs no further treatment and is ready to be made up to the desired volume for the pipette method. The whole operation takes less than 2 hours, and the number of soils that can be treated at the same time is limited only by the number of beakers that can be heated at one time.

As regards the comparative merits of Na and Li hydroxides as dispersing agents, the author is inclined to favor the latter, which has better dispersing power than the former. Another point in favour of LiOH is that the actual weight of the chemical added to the soil is less in this case than in the case of NaOH.

For soils containing large quantities of gypsum and soluble salts the method is modified as follows:—

After the first boiling with N (NH$_4$)$_2$CO$_3$, about 100 cc. of 2 N (NH$_4$)$_2$CO$_3$ is added to the suspension and filtered through a Buchner funnel on which a filter paper is stuck with molten paraffine wax. The soil is then leached once only with 100 cc. of 0.2 N (NH$_4$)$_2$CO$_3$. It is then taken up with about 250 cc. of water, boiled for 10 minutes, and 4 to 8 cc. of NaOH or LiOH is added and boiling is continued till the volume is reduced to one half. The suspension is then ready for making up to the desired volume for the pipette method.

It might be mentioned that the addition of 8 cc. of N NaOH or LiOH is not necessary for all soils, in fact the majority of soils require only 4 to 5 cc. Approximately 1.3 cc. N alkali is required for every gram of clay, and 8 cc. is the upper limit required by red ferruginous, or black cotton, soils.

EXPERIMENTAL

Altogether 88 soils were examined. These comprised practically all the types found in India and were specially selected out of a larger collection so as to include the largest available number of those soils that had failed to give

TABLE 1

Clay content of ordinary agricultural soils

SOIL NO. P. C.	LOCALITY	pH VALUE	CARBONATES	CLAY	
				NaCl method	(NH₄)₂CO₃ method
			per cent	per cent	per cent
1	Pusa (Bihar)	8.45	37.05	11.3	13.7
4	Ranala (Punjab)	8.55	0.98	15.2	18.1
5	Ranala (Punjab)	8.77	1.64	12.3	14.8
8	Coimbator I (Madras)	8.41	0.65	25.2	25.7
10	Coimbator II (Madras)	8.71	1.08	35.6	37.0
11	Coimbator III (Madras)	8.77	2.48	32.8	31.0
17	Kalyanpur (U.P.)	8.20	0.78	11.9	17.5
19	Mandalay (Burma)	8.40	1.54	42.4	44.2
21	Kaing (Burma)	8.25	3.31	13.5	16.4
23	Myetha (Burma)	7.41	0.28	11.3	11.8
24	Meerut (U.P.)	8.59	2.02	8.0	11.3
26	Hebbel (Mysore)	8.11	0.14	22.6	20.5
28	Nagpur (C.P.)	8.38	1.37	44.6	44.6
31	S. Travancore	8.01	3.12	22.8	28.1
34	Gurdaspur (Punjab)	7.21	0.12	11.3	15.5
35	Gurgaon (Punjab)	7.98	0.12	18.3	18.3
36	Hansi (Punjab)	8.45	0.52	11.7	14.4
43	Churland (Bihar)	8.41	5.87	19.7	24.7
44	High Land (Bihar)	8.54	7.46	8.4	13.2
48	Montgomery I (Punjab)	8.55	6.24	19.8	24.8
50	Tarnab I (N.W.F.P.)	8.54	16.95	17.7	19.6
51	Tarnab II (N.W.F.P.)	8.68	17.00	12.9	13.9
52	Sabour (Bihar)	8.02	0.18	11.3	10.0
54	Montgomery II (Punj.)	8.71	3.16	3.2	6.2
55	Sakrand I (Sindh)	8.36	11.32	11.0	14.9
56	Sakrand II (Sindh)	8.53	11.20	13.1	17.5
59	Raghopur (U.P.)	8.83	0.50	10.9	12.2

maximum dispersion with the author's NaCl method. For convenience of reference the soils were grouped as follows:

1. Ordinary agricultural soils.
2. Black cotton soils.
3. Laterite soils.
4. Humus soils.
5. Alkali soils.
6. Soils containing soluble salts and gypsum.
7. International soils.

1. *Ordinary agricultural soils.*—This group included all soils which had no striking feature to impart them distinct individuality, but were picked up at random to represent an average type of the usual agricultural soils. The

TABLE 2

Clay content of black cotton soils

SOIL NO. P. C.	LOCALITY	pH VALUE (H)	CARBONATE	CLAY	
				NaCl method	(NH₄)₂CO₃ method
			per cent	per cent	per cent
2	Akola (Bombay)	8.21	5.22	59.3	62.8
3	Dharwar (Bombay)	7.64	0.15	62.2	63.8
13	Nagpur (C.P.)	8.53	2.06	58.9	60.4
27	Babbur (Mysore)	9.03	5.08	53.2	59.1
29	Jabbalpur (C.P.)	8.05	0.32	63.0	57.4
30	Hoshangabad (C.P.)	8.45	3.30	54.1	55.1
38	Broach (Bombay)	8.29	0.22	52.9	51.9
41	Poona (Bombay)	8.74	7.73	53.4	61.6
42	Khandesh (Bombay)	9.00	4.81	53.4	58.9
46	Baroda (Bombay)	7.63	0.36	56.4	59.0

TABLE 3

Clay content of lateritic and ferruginous soils

SOIL NO. P. C.	LOCALITY	pH VALUE (H)	CLAY	
			NaCl method	(NH₄)₂CO₃ method
			per cent	per cent
6	Dacca (Bengal)	5.29	28.4	31.6
9	Malabar (Madras)	5.76	21.6	22.8
12	Tocklai (Assam) Composit.	5.83	3.8	14.8
14	Estate Soil (Mad.)	5.37	11.1	31.5
15	Shillong (Assám)	7.71	19.4	27.6
18	Mandalay (Burma)	5.79	19.9	25.2
20	Lower Burma	5.64	6.5	15.1
22	Pyinana Soil (Burma)	6.85	15.2	17.8
25	Bhur Soil (U.P.)	7.40	4.0	5.8
32	Thuravoor (Tranvanc.)	5.17	62.9	72.5
37	Rangpur (Bengal)	7.65	13.1	17.0
40	Umareth (Bombay)	7.45	10.7	14.0
45	Bihar	6.33	27.3	32.6
49	Madhupur (Bihar)	5.72	5.9	15.3

results given in table 1 show that although NaCl method is quite satisfactory for these soils, the ammonium carbonate method gives slightly better dispersion.

2. *Black cotton soils.*—The characteristic features of these soils are their dark color, high clay content, and high base exchange capacity. They respond

to the NaCl as well as the $(NH_4)_2CO_3$ method. This will be seen from table 2, which records the clay content for these soils by the two methods along with other relevant data.

3. *Laterite soils.*—This group included all the base unsaturated red and yellow soils. The NaCl method failed to give maximum dispersion with some

<div align="center">TABLE 4</div>

Clay content of humus soils by the international and ammonium carbonate methods

SOIL NO.	pH VALUE	LOSS ON IGNITION	CLAY	
			International (B)	Ammonium carbonate
		per cent	per cent	per cent
265	6.49	11.1	13.7	15.9
266	6.36	9.77	13.8	15.5
267	6.10	3.19	9.7	12.5
346	5.95	5.66	11.5	10.6
350	5.90	6.07	12.1	21.3
351	6.08	7.5	17.4	18.1
352	6.31	4.06	18.0	20.8
395	5.58	6.87	29.4	28.8
190	5.07	8.25	14.0	13.5
S.30	5.40	26.9	41.9	49.3
S.34	5.10	16.5	43.8	43.6
S.58	6.00	14.8	43.6	39.6
S.64	5.70	10.8	19.5	22.6

<div align="center">TABLE 5</div>

Clay content of alkali soils

SOIL NO. P. C.	LOCALITY	pH VALUE	CARBONATE	CLAY	
				NaCl method	$(NH_4)_2CO_3$ method
			per cent	per cent	per cent
7	Punjab	9.58	5.62	21.8	26.4
39	Bombay	9.11	1.46	8.9	15.3
47	Punjab	9.98	6.60	17.1	22.4
53	Punjab	9.87	6.96	17.6	17.9
57	U.P.	10.40	0.80	4.4	7.2
58	U.P.	10.36	3.45	13.4	10.0
60	U.P.	10.19	3.45	7.4	8.7

of them. The results given in table 3 show the superiority of the $(NH_4)_2CO_3$ method over the NaCl method.

4. *Humus soils.*—This group included all those soils with which the NaCl method had failed. The values in this case are compared with the International B method which is known to give maximum dispersion with such soils. The results are recorded in table 4 and show that the ammonium

carbonate method gives as good a dispersion as the International method except in one case where it is distinctly better. The last four soils in this group were kindly supplied by Dr. Sen of Dacca and were classed as lateritic. The revelant data about these soils has been taken from the paper by Chakraborty and Sen (1). The clay content of the last four soils is on the oven-dry basis; and in the case of the remaining soils the percentage of the ignited clay fraction is given.

TABLE 6

Clay content of soils containing soluble salts and gypsum

SOIL NO.	SOLUBLE SALTS	CLAY	
		NaCl method	(NH₄)₂CO₃ method
	per cent	*per cent*	*per cent*
H.1	15.85	3.3	11.5
H.2	35.37	2.5	7.9
H.3	15.97	16.7	20.9
H.4	24.75	22.0	21.8
H.5	15.48	20.0	21.2
H.6	7.87	10.0	14.7
H.7	14.88	11.8	14.5
H.8	10.05	11.5	15.1
H.9	2.10	20.8	25.9
H.10	48.51	12.9	15.0
H.11	18.51	10.5	32.3
H.12	16.18	16.2	18.3

TABLE 7

Clay content of international soils

	NaCl method	(NH₄)₂CO₃ method
	per cent	*per cent*
Rendzina soil Cejě....................................	26.8	33.9
Podsol soil Zdâr.......................................	22.0	29.8
Soil sample from Zagreb.............................	28.0	31.3
Budapest soil..	32.5	33.8
Badob soil...	66.5	64.0

5. *Alkali soils.*—The characteristic features of these soils are their high pH values and the presence of exchangeable Na. Some of them contain large amounts of free Na_2CO_3 (soils 57, 58, and 60 in table 5). Free Na_2CO_3 is leached out in the NaCl method but appears as clay in the $(NH_4)_2CO_3$ method. When the percentage of soluble salts in these soils is high the modified technique is recommended. The results given in table 5 show that the $(NH_4)_2CO_3$ method is as satisfactory for these soils as is the NaCl method. As a rule

alkali soils, unless they contain gypsum, do not present any difficulty in dispersion, and even simple shaking with water is sufficient to bring about maximum dispersion for most of them.

6. *Soils containing soluble salts and gypsum.*—It is obvious that soluble salts and gypsum would cause flocculation of the soil unless they were leached out. Besides, in the pipette method the soluble salts would appear as clay, which is not desirable. Because of its limited solubility, gypsum is leached extremely slowly. In the modified technique of the ammonium carbonate method, all the gypsum is converted into calcium carbonate and ammonium sulfate. The latter can be easily leached with a single washing. The results of a comparison between the NaCl and $(NH_4)_2CO_3$ method are given in table 6.

7. *International soils.*—These soils were received by the author some time ago, in connection with the co-operative work on the methods of dispersing soils for mechanical analysis. Some of these soils will be familiar to other workers. A comparison of the clay percentage obtained by the $(NH_4)_2CO_3$ and the NaCl methods (table 7) shows the superiority of former over the latter. A reference to the results of the co-operative work given in the *Proceedings of the International Congress of Soil Science* (3) also shows that the ammonium carbonate method gives the maximum clay obtainable by any method.

CONCLUSION

In view of the importance attached to the methods of dispersing soils for mechanical analysis by the International Society of Soil Science, it is necessary that the ammonium carbonate method be given an exhaustive trial. It can not be called too drastic because boiling is already a standard procedure in the International method. The author has attempted to include all types of soils available, but the possibility of others presenting peculiar difficulties is not remote. The ideal method that would succeed with all types of soils (and of soil scientists) may still await discovery, but the present method seems to be a step in the right direction.

SUMMARY

The ammonium carbonate method of dispersing soils for mechanical analysis has been described. It consists in boiling the soil with N ammonium carbonate solution and continuing the boiling after the addition of some NaOH or LiOH. The method has been shown to give maximum dispersion with all types of soils including laterite and humus.

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A SIMPLER METHOD OF EXPRESSING THE MECHANICAL ANALYSIS OF MANY COMMON SOILS*

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For many years a mechanical analysis (or statement of the percentage proportions of sand, coarse silt, fine silt, coarse clay, fine clay, etc.) has been made of nearly every soil studied for agricultural purposes. The analysis is usually made by shaking up a soil with water and allowing the soil particles to settle. The sand grains, which are larger in diameter than 1/20 mm., fall in the standard cylinder in 40 seconds. At the end of an hour the silt (or particles between 1/200 mm. and 1/20 mm.) have fallen to join the sands on the bottom of the cylinder. Two hours after the cylinder has been set down, the coarse clay has fallen, and so on.

Some analysts divide the soil particles into four size groups; others, into seven. Where seven groups are used the operator waits till a given time and then withdraws a certain volume of the muddy liquid, evaporates and weighs this, and calculates the weight of soil in suspension in the whole column at that particular time. This is performed seven times and constitutes a very tedious and slow process. In 1926, Bouyoucos designed a soil hydrometer which was graduated by comparing it with 30 soils analyzed by the ordinary method, so providing a simple and accurate method, which is gaining in favor. For seven groups the hydrometer is placed in the liquid, read, and removed at six particular times.

In the course of a research on "The Identification of Soils in Civil Engineering" it became necessary to analyze mechanically a number of soils with as much accuracy as possible. The soils came from Otago, Canterbury, and Auckland Provinces and were of widely different origin. On its receipt at the laboratory each soil was air dried, passed through a ⅛-inch sieve, well mixed, and then stored in an air-tight cannister. The soils referred to in this paper were those which passed through a ⅛-inch sieve without any appreciable amount being retained. From four to eight samples of each soil were analyzed by the hydrometer method, allowance being made for moisture content and for temperature. Just before analysis, dispersion was effected by the standard elec-

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tric stirrer method. The method of dispersion was checked many times by shaking samples end over end for 48 hours on a wheel.

When all the points for each soil were plotted on a graph of *percentage of soil in suspension* against *time of hydrometer reading*, a smooth curve was obtained.
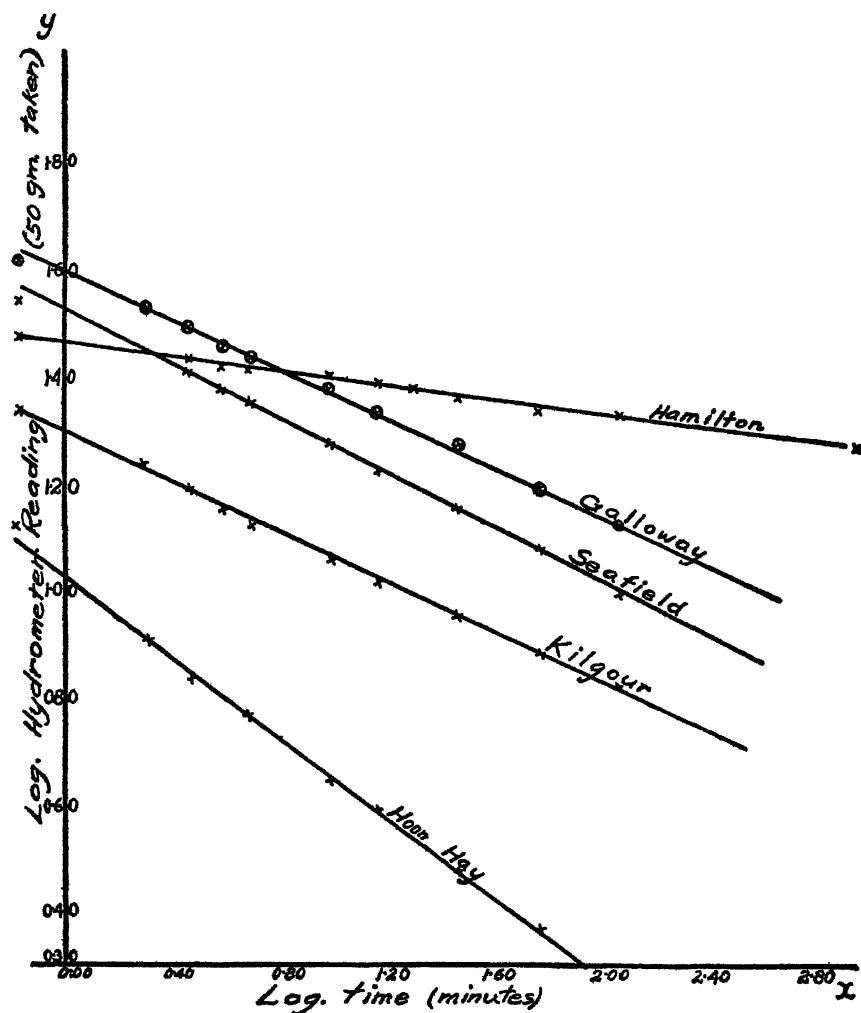

FIG. 1. SOME TYPICAL LINES FOR SOILS WHEN LOGARITHM OF TIME OF HYDROMETER READING IS PLOTTED AGAINST LOGARITHM OF HYDROMETER READING

There was a different curve for each soil, and in every case the curve was very smooth. It was then found that the smoothness of soil curves had been remarked on by G. W. Robinson (2). This encouraged the writer to plot the *logarithm of the percentage in suspension* against the *logarithm of the time of hy-*

drometer reading. In 13 cases out of 14 the result was a straight line. The exception was Glentunnel fire clay, a very hard fine-grained clay which might be counted perhaps a rock rather than a soil.

Readings taken at 40 seconds did not always conform well to the line. When the cylinder is first set down a swirling motion occurs, froth obscures the graduations, and settling occurs so rapidly that an error of a few seconds may cause a large error in the readings.

There was a different straight line for each soil, soils of similar texture having their lines close together. In geometry it is shown that such a straight line is completely described with respect to two axes of reference (lines at right angles such as those along which *log percentage in suspension* and *log time of reading* were measured), if we measure the slope of the line and measure where it cuts one of the axes. In other words, the whole result of the mechanical analysis can be expressed by two numbers for the particular soil. Typical lines are shown in figure 1.

TABLE 1

The means of eight hydrometer analyses of Hamilton soil (corrected for moisture and temperature)

TIME (T) OF READING HYDROMETER	CORRECTED HYDROMETER READING H (50 GM. TAKEN)	LOG T	LOG H
40 seconds	29.5	$\overline{1}.824$	1.470
2 minutes	28.0	.301	1.447
3 minutes	26.9	.477	1.430
4 minutes	26.1	.602	1.417
5 minutes	26.0	.699	1.415
10 minutes	25.1	1.000	1.400
15 minutes	24.3	1.176	1.386
30 minutes	22.9	1.477	1.360
1 hour	21.6	1.778	1.335
2 hours	21.3	2.079	1.328
16 hours	18.3	2.982	1.263

Example

Column 3 (table 1) is plotted against column 4 and a fair line through the points is found to be a straight line cutting the y axis (along which log hydrometer reading is measured) at the value 1.460. In geometry this value would be termed the "intercept." From antilogarithm tables 1.460 is found to be the logarithm of 28.84 (which would be the hydrometer reading at 1 minute). Since in this work 50 gm. of soil was taken, $28.84 \times 2 = 57.68$ is the percentage of material in suspension at 1 minute.

Next the slope or "gradient" of the line must be found. From the graph it will be seen that the line drops from the value 1.460 to the value 1.323 while log time changes in value from 0.000 to 2.079.

$$\therefore \text{Gradient} = \frac{1.460 - 1.323}{0.000 - 2.079} = \frac{0.137}{-2.079} = -0.066$$

That is, the numbers 57.68 and 0.066 express the mechanical analysis of Hamilton soil. The writer proposes that these two quantities be named "fine material" and "settling rate" respectively. From these two numbers the straight line can be reproduced at any time, and the amount of fine silt, coarse clay, etc. can be read off.

Theory

Co-ordinate geometry shows that the equation of a straight line, plotted by x and y co-ordinates, is $y = mx + c$ where m is a constant called the "gradient" (or tangent of the angle made with the x axis) and c is a constant called the "intercept" (or value of y when $x = 0$)

In this work $\qquad$ $\text{Log } H = m \text{ (Log. } T) + c$

e.g. Hamilton $\qquad$ $\text{Log } H = -0.066 \text{ Log } T + 1.460$

i.e. $\qquad$ $\text{Log } H = \text{Log } (T^{-0.066}) + \text{Log } 28.84$

$$= \text{Log } (28.84 \ T^{-0.066})$$

$$= \text{Log } \frac{28.84}{T^{0.066}}$$

or $\qquad\qquad\qquad\qquad H = \dfrac{28.84}{T^{0.066}}$

Whence $\qquad$ $P \text{ (percentage in suspension)} = \dfrac{57.68}{T^{0.066}}$ is the equation of the settling curve for Hamilton.

TABLE 2

Complete mechanical analysis of certain soils

SOIL	FINE MATERIAL	SETTLING RATE
Hamilton	57.68	0.066
Galloway	78.72	0.234
Seafield	67.0	0.264
Horarata	63.9	0.144
Hoon Hay	21.2	0.392

An analysis expressed in terms of "fine material" and "settling rate" is readily convertible into the old nomenclature and vice versa.

The complete mechanical analysis of certain soils may be stated as in table 2.

The writer plotted the results given by Bouyoucos (1) and found that 13 out of the 15 Michigan soils gave lines which were straight within the 2 to 3 per cent which could be expected as experimental error.

The results of 14 samples of the Swan Hill Irrigation District, Victoria (3) were obtained for fine silt, coarse silt, and clay and were plotted logarithmically as before. The mid point (fine silt) deviated from the straight line joining the other two points by an amount within the limit of experimental error in each case. This was all the more remarkable since the official method used requires readings to be taken at a different depth at each pipetting. This, combined with the greater use of sieving, might be expected to mask any regular gradation in a soil. However, the official method is used very extensively.

The general result may be summarized as follows: Nearly every soil (material passing ⅛-inch sieve) exhibits a regular grading in the size of its particles from coarse to fine. Further, this grading, in most cases, is such as to permit the mechanical analysis of each soil to be represented logarithmically by a straight line to an accuracy within the limits of experiment and well within the limits demanded by the agriculturist and engineer.

Application

It is easier to classify soils according to two properties than according to seven and it is easier to take two or three readings than seven. Although readings of the hydrometer taken at 5 minutes, 15 minutes, and 30 minutes would give the straight line and the complete mechanical analysis in a quarter of the usual time it is obviously unwise to shorten the analysis until a great deal of confirmatory work has been done. Exceptions are bound to occur (perhaps with pure clays or pure silts), and these may not always be easily recognizable. Again a soil could be made up to show an irregular settling curve. However, if the mechanical composition of only a quarter of the world soils (this paper records 26 out of 29 examined) can be expressed completely by the quantities "fine materials" and "settling rate" so that they are more capable of practical interpretation in their relation to tilth, water-holding capacity, suitability for drainage and irrigation, strength in foundations, porosity in dams, etc., the result is a very tangible one.

One example will be sufficient to make this clear.

The values	sand fine silt coarse silt fine clay coarse clay organic matter grain shape ·	all appear to have an effect upon foundation strength

Although each value, such as sand, grain shape, etc., can be measured, it is impossible to combine all these to predict a foundation strength. If the factors are reduced to

fine materials settling rate organic matter shape of grains	and foundation strength,

a working rule becomes more possible. The same simplification applies to many other soil effects.

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A STUDY OF PHOSPHORUS PENETRATION AND AVAILABILITY IN SOILS[1]

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Investigation by soil scientists during the past two decades has shown that phosphorus fertilizers in general become largely unavailable to plants at the place where they come in contact with the soil. A few studies have shown that phosphorus carriers applied on permanent pasture sod penetrate only about one inch or less during the first year, and very little penetrates into the third inch even after 5 years.

The comparative effect of various fertilizer treatments on phosphorus penetration in pastures when the fertilizers are applied regularly over a period of more than a few years has not been studied. The purpose of the present study is to show the penetration of phosphorus under the aforementioned conditions and the relative penetration of rock phosphate and superphosphate under various conditions in the laboratory.

The literature dealing with phosphorus fixation dates back to 1850, when Way (19) demonstrated by simple percolation experiments that phosphate of soda dissolved in water and calcium phosphate dissolved in dilute sulfuric acid lost all of their detectable phosphates when passed through a soil. In more recent years the work of Crawley (4), Schreiner and Failyer (13), Fraps (5), Fudge (6), and Gaardner (7) show, not only that phosphorus is fixed by soils almost at the point at which it comes in contact with the soil particles, but also that clay soils fix phosphorus more rapidly than do sandy soils. Iron, aluminum, and calcium are the main elements responsible for phosphorus fixation, and the effect of nitrogen fertilizers on phosphorus fixation is due to change in soil reaction or introduced cations.

The comparison of rock phosphate and superphosphate as sources of phosphorus for plants has resulted in some conclusions which do not agree. Mooers (9), Roberts (12), and Conner (3) find that rock phosphate produces as good results as superphosphate and often better results under certain conditions.

277

Bartholomew (1) and Thorne (15) have shown that superphosphate is a more efficient carrier of phosphorus than is rock phosphate.

By working superphosphate into knife grooves in old established bluegrass sod, Midgley (8) increased the yield of the grass 71.5 per cent over surface applications of the same amount of phosphorus.

The study by Brown and Munsell (2) shows that 20 months after the surface application of phosphatic fertilizers on pastures neither rock phosphate nor superphosphate treatment shows marked increases in soluble phosphorus below the 1-inch layer.

PLAN OF EXPERIMENTS

In the present study the main object was to get further information on the factors concerned in the penetration and availability of phosphorus in permanent pasture soils. The permanent pasture plots at the Snowshoe and Bradford County experiment fields offered excellent opportunity for this phase of the study. The available phosphorus and the pH of several depths in these plots were used as an indication of the most profitable additional study.

The additional study was divided into two parts; namely, first, an investigation of the factors concerned in the relative penetration of rock phosphate and superphosphate under carefully controlled laboratory conditions; the second, the determination of the effect of field applied phosphorus fertilizers on the phosphorus content of soils and plants.

METHODS OF ANALYSIS

Available phosphorus was determined on the soil samples by the method recommended by Truog (17). Throughout this discussion "available phosphorus" refers to the amount determined by this method.

The pH of the soils was determined electrometrically with the quinhydrone electrode.

A slight modification of the colorimetric method suggested by Pfeilsticker (11) was used to determine total phosphoric acid of the plants.

EXPERIMENTAL

Phosphorus penetration in field plots

Previous phosphorus penetration studies (14, 18, and 8) indicated that most of the available phosphorus applied on permanent pastures remained very near the surface. For this reason the upper 5 inches were sampled by one-half inch layers, the next 3 inches by inch layers, and the 8- to 12-inch depth was taken in two 2-inch layers. The samples were taken in November, 1932, from Snowshoe field B and Bradford County field B plots.

Soil samples from the plots were transferred to the laboratory, air dried, sieved through a 20-mesh sieve, and stored for 1 year before any analyses were made.

Table 1 shows the yields, fertilizer treatments, and amounts of available phosphorus of the Snowshoe plots. Fertilizer treatments date back 17 years prior to sampling.

Table 1 shows that most of the available phosphorus in plot 2, a permanent pasture plot which received no phosphatic fertilizer, is concentrated in the upper one-half inch of the soil.

TABLE 1

Yields, fertilizer treatments, and available phosphorus of Snowshoe field B plots

	PLOT 2	PLOT 3	PLOT 4	PLOT 5	PLOT 6	PLOT 7	PLOT 8	PLOT 9
Treatment*	Ca	Ca, P	Ca, R	Ca, P, K	None	Ca, N, P, K	Ca, M, P	Ca, M, R
Yield (lbs. per acre):†								
Weeds.............	596	54	101	135	443	338	115	140
Sweet clover.......	66	426	794	634	....	368	515	1,236
Pasture grasses.....	96	2,071	1,862	2,521	....	3,530	2,488	2,127
Depth, inches	*p.p.m. P*	*p.p.m. P*	*p.p.m. P*	*p.p.m. P*	*p.p.m. P*	*p.p.m. P*	*p.p.m. P*	*p.p.m. P*
0–½	12	30	132	26	8	12	13	96
½–1	5	24	142	17	5	13	12	99
1–1½	5	18	134	14	3	10	9	95
1½–2	5	12	136	11	<2	9	8	84
2–2½	4	7	126	8	<2	11	7	93
2½–3	5	6	124	8	<2	10	6	81
3–3½	5	4	87	6	<2	4	3	69
3½–4	4	3	72	4	<2	3	2	59
4–4½	4	3	79	4	<2	4	<2	53
4½–5	3	4	56	5	<2	4	3	41
5–6	2	5	40	5	<2	4	3	24
6–7	2	4	39	4	<2	2	2	13
7–8	2	3	10	4	<2	2	2	4
8–10	2	3	4	4	<2	3	3	5
10–12	..	2	4	4	<2	<2	<2	5

* Ca—lime to equal Vietch lime requirement at start of experiment.
P—65 pounds P_2O_5 as superphosphate every second year.
R—260 pounds P_2O_5 as rock phosphate every second year.
K—100 pounds muriate of potash every second year.
N—300 pounds nitrate of soda every second year.
M—4 tons manure every second year.
† Average annual yield (air dry basis) according to White and Holben (20).

Plot 3 shows that very little of the superphosphate remains available and none of it penetrates deeper than 3 inches. It is impossible to say how much, if any, of the phosphorus penetrated, since the first application was harrowed in. Under these circumstances it is probable that phosphorus would be worked into the first 3 inches mechanically.

Comparison of plot 3 and 4 shows the remarkably larger amounts of available phosphorus on the rock phosphate treated plot when compared to the superphosphate treated plot. Likewise available phosphorus has penetrated to a depth of more than 7 inches where applied as rock phosphate.

TABLE 2

pH of soil samples from Snowshoe field B plots

DEPTH	PLOT 2	PLOT 3	PLOT 4	PLOT 5	PLOT 6	PLOT 7	PLOT 8	PLOT 9
inches								
0–½	5.89	5.55	5.97	5.36	4.75	5.68	5.86	5.89
½–1	6.16	5.66	6.14	5.43	4.56	5.66	5.78	6.02
1–1½	5.87	5.88	6.39	5.45	4.58	5.63	5.79	6.11
1½–2	5.87	5.95	6.42	5.60	4.56	5.67	6.11	6.23
2–2½	6.03	5.82	6.39	5.51	4.49	5.68	6.13	6.22
2½–3	5.75	5.66	6.54	5.31	4.60	5.77	5.98	6.31
3–3½	5.67	5.49	6.46	5.21	4.70	5.76	6.06	6.09
3½–4	5.43	5.23	6.44	5.17	4.72	5.73	6.05	6.16
4–4½	5.42	4.95	6.23	5.00	4.74	5.69	5.74	6.03
4½–5	5.25	5.12	5.97	5.22	4.75	5.53	5.60	5.92
5–6	5.05	4.91	5.73	5.06	4.79	5.40	5.59	5.81
6–7	4.95	4.91	5.86	4.89	4.85	5.28	5.54	5.43
7–8	5.05	4.73	5.44	4.93	4.93	5.07	5.30	5.18
8–10	4.74	4.58	5.15	4.67	4.72	4.89	5.09	4.94
10–12	....	4.53	4.72	4.61	4.69	4.73	4.94	4.79

TABLE 3

Available phosphorus and pH of plots 1, 2, 3 and 4 of field B at the Bradford County experiment

	PLOT 1	PLOT 2	PLOT 3	PLOT 4
Treatment............	None	Lime	Superphosphate	Lime + superphosphate
Yield (lbs. per acre):*				
Weeds..............	1,519	722	1,261	351
Sweet clover........	23	858	.....	828
Pasture grasses......	162	1,402	506	1,831
Depth, inches	*p.p.m. P*	*p.p.m. P*	*p.p.m. P*	*p.p.m. P*
0–½	29	18	80	70
1–1½	7	6	40	44
2–2½	3	5	18	23
3–3½	3	4	7	11
4–4½	4	4	4	5
10–12	3	3	3	3
	pH	*pH*	*pH*	*pH*
0–½	5.70	6.51	5.58	5.95
1–1½	5.08	6.61	4.96	5.94
2–2½	5.01	6.40	4.92	5.81
3–3½	5.00	6.10	4.79	5.68
4–4½	5.02	6.02	4.77	5.59
10–12	5.07	5.15	4.65	4.93

* Average annual yield (air-dry basis) according to White and Holben (20).

Plots 8 and 9 produced higher yields than the corresponding plots 3 and 4, which did not receive manure, but the available phosphorus did not penetrate deeper in plots 8 and 9 than where manure was not used. Available phosphorus was somewhat lower in these plots than in plots receiving no manure.

This may be caused by the absorption of more phosphorus by the plants on plots receiving manure.

These results agree well with part of the results by Brown and Munsell (2). They found a great deal more available phosphorus in plots treated with rock phosphate than in those treated with superphosphate. However, they found no more penetration of phosphorus from rock phosphate than from superphosphate. The greater penetration of available rock phosphate shown in the present study may be caused by a longer term of fertilizer treatment than that represented in Brown and Munsell's work.

Apparently there is no significant difference in phosphorus penetration and availability between plots 5 and 7. This is contrary to the findings of Midgley (8), who reports greater phosphorus penetration where sodium nitrate is applied with superphosphate than where sodium nitrate is not applied.

Since phosphorus availability is often correlated with soil reaction, the pH of the soil samples from Snowshoe was determined. Results of these determinations are given in table 2.

Table 2 shows that the only consistent difference between plots that received limestone is the more alkaline reaction of the plots receiving their phosphorus in the form of rock phosphate.

Results of the determination of available phosphorus and of pH of the Bradford County soil samples verified the correctness of the results on the Snowshoe soil samples.

Table 3 shows the yields, fertilizer treatment, available phosphorus, and pH of plots 1, 2, 3, and 4 of the Bradford County field B experiment. The data indicate that there is no significant difference in the available phosphorus content of a superphosphated soil when limed and when not limed. Also practically all of the available phosphorus remains in the upper $3\frac{1}{2}$ inches of the soil. The consistently more acid reaction of plots receiving superphosphate over those receiving no phosphorus is the most significant fact shown by the pH values in table 3. This may be caused by the crops on phosphorus treated plots absorbing more calcium and other bases from the soil, which leaves the soil more acid.

Laboratory percolation studies

The results shown in table 1 indicate that rock phosphate solubility in soils and the crop response from it can not be correlated. That is, although rock phosphate plots show more available phosphorus than do superphosphate plots, crop response shows that superphosphate is the more efficient fertilizer. Additional information comparing rock phosphate and superphosphate therefore seemed desirable.

The laboratory percolation tests were conducted in glass percolation tubes. The bottom hole of each tube was covered with cheesecloth, and pulped filter paper in water was placed on top of the cloth to form a tight fitting filter pad (fig. 1, D). Three hundred grams of stock soil (fig. 1, C) was placed in the

tube. The upper filter pad where present (fig. 1, B) was made by pouring filter paper in water on top of the soil.

The stock soil used in all percolation tests was Morrison sand, subsoil. This soil is almost free of available phosphorus, water penetrates it rapidly, there is very little exchangeable calcium in it, and it is a residual soil formed from a high iron content, coarse-grained sandstone.

In most of the tests four times as much phosphorus was applied in the form of rock phosphate as was applied as superphosphate. These are the amounts applied on the Snowshoe field B plots and many other field tests of the two phosphorus carriers.

The fertilizer was mixed with 40 to 50 gm. of the stock soil and clean quartz sand (fig. 1, A). Distilled water for leaching was supplied through a system of syphons. The water dripped on the filter paper on top of the soil at a con-
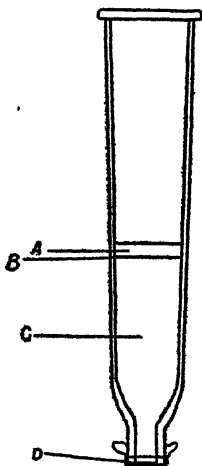
FIG. 1. PERCOLATION TUBE SETUP USED IN LABORATORY PENETRATION TESTS

trolled rate. The object of this was to make the water application as near like a continuous rain as possible. The penetration of phosphorus by this method was no doubt greater than under field conditions. However, comparative penetration between treatments was considered more like that obtained under field conditions than if the soil were flooded by additions of several inches of water at a time.

All percolation tests were run in duplicate. The averages of duplicates are reported here. The object of the first percolation test was to determine whether phosphorus penetrated the soil entirely as a solution or partially as a suspension of small particles. In this test half of the percolation tubes were fitted with filter pads between fertilizer and stock soil (fig. 1, B) and the others had no obstruction to the movement of the phosphorus carrier in suspension. Distilled water was slowly dripped on the soils, as previously described, for

about 144 hours. At the end of this time 65 inches of water had passed through the soils. The results of this first percolation test are shown in table 4.

Table 4 shows that preventing the movement of superphosphate in suspension has very little effect on the penetration of phosphorus that is available. In the rock phosphate treated soils, however, roughly three times as much available phosphorus had penetrated below the layer in which it was mixed where the filter pad was absent as where it was present. Analysis of the filter pads showed that immaterial amounts of phosphorus were absorbed by them. These data would lead us to believe that probably a large part of the penetration of rock phosphate at Snowshoe was caused by the fertilizer being carried down as particles in suspension.

TABLE 4

Available phosphorus and pH of Morrison subsoil from percolation tubes after leaching with 65 inches of distilled water

TREATMENT*	SURFACE† 50 GM.		NEXT 100 GM.		2ND 100 GM.		3RD 100 GM.		LEACHATE
	$\frac{mgm.}{P}$	pH	$\frac{mgm.}{P}$	pH	$\frac{mgm.}{P}$	pH	$\frac{mgm.}{P}$	pH	$mgm.\ P$
None........................	0.18	5.59	0.25	5.45	0.30	5.35	0.26	5.34	None
Superphosphate 400‡.................	1.57	5.76	1.42	5.67	0.75	5.61	0.16	5.40	0.008
Rock phosphate 915‡...............	17.03	6.35	0.56	5.99	0.23	5.19	0.23	5.22	0.070
Superphosphate 400.................	1.75	5.49	1.35	5.52	0.84	5.46	0.46	5.39	0.050
Rock phosphate 915.................	13.00	6.33	1.70	5.94	0.52	5.39	0.38	5.27	0.100
Superphosphate 400, NaNO₃ 320.......	1.65	5.80	1.76	5.58	0.80	5.49	0.48	5.31	None
Superphosphate 400, (NH₄)₂SO₄ 233.....	1.38	5.52	1.42	5.51	0.71	5.46	0.43	5.39	0.030
Rock phosphate 915, NaNO₃ 320.......	12.90	6.12	0.76	5.76	0.40	5.35	0.25	5.27	0.190
Rock phosphate 915, (NH₄)₂SO₄ 233.....	14.60	6.30	0.69	5.56	0.30	5.42	0.18	5.11	0.213

* Fertilizer used and pounds per acre.
† Fertilizer mixed with this layer prior to leaching.
‡ Filter pads placed just under the surface 50 gm.

The percolate from sodium nitrate plus superphosphate treated soil was considerably lower in phosphorus than the percolate from soil receiving superphosphate only. Sodium nitrate had little, if any, effect on the penetration of rock phosphate but allowed more to be carried into the percolate. These data do not agree closely with the findings of Midgley (8), who has shown superphosphate to be considerably more soluble when applied with sodium nitrate than when applied alone.

After the comparisons of rock phosphate and superphosphate in soils made thus far, rock phosphate was applied in sufficient quantities to furnish four times as much total phosphoric acid as the superphosphate applications. Also the laboratory percolation tests were on soils of pH about 5.4. It seemed advisable, therefore, to test the effect of some treatments which contained the same amounts of phosphorus from superphosphate and from rock phosphate. Table 5 gives the results of this percolation study.

Superphosphate, in comparison to rock phosphate, has a great deal more phosphorus soluble at a pH near neutrality than at pH 5.5, as shown in tables 4 and 5. When superphosphate was applied to equal the phosphoric acid strength of the rock phosphate application in a nearly neutral soil, about 50 per cent more phosphorus was available in the rock phosphate soil than in the superphosphate soil after leaching both with 50 inches of distilled water. However, table 5 shows that superphosphate penetrates the soil much more readily than does rock phosphate under these conditions.

The effect of soil reaction on superphosphate and rock phosphate penetration and availability in a soil was tested by bringing the Morrison sand subsoil of pH 5.03 to various pH values by the use of lime or sulfuric acid. The fertilizer was mixed with 40 gm. of the stock soil containing 20 per cent white sand. The mixture was applied on the surface of the soil in the percolation tubes. The soil·was continuously leached with distilled water for 4 days,

TABLE 5

Available phosphorus and pH of limed Morrison subsoil from percolation tubes after leaching with 50 inches of distilled water

TREATMENT*	SURFACE† 45 GM.		NEXT 100 GM.		2ND 100 GM.		3RD 100 GM.		LEACHATE
	$\frac{mgm.}{P}$	pH	$\frac{mgm.}{P}$	pH	$\frac{mgm.}{P}$	pH	$\frac{mgm.}{P}$	pH	$mgm.$ P
None......................	0.09	6.82	0.30	7.06	0.30	7.38	0.30	7.42	None
Superphosphate 400...........	0.30	6.38	1.10	6.57	1.20	7.18	1.00	7.10	0.090
Superphosphate 1,600..........	1.90	6.17	3.00	6.76	3.70	6.94	3.20	7.25	0.188
Rock phosphate 915...........	13.10	6.86	4.30	7.46	0.60	7.40	0.40	7.39	None

* Fertilizers and pounds per acre.
† All fertilizer was mixed with this layer which was 27 per cent limed Morrison subsoil and 73 per cent washed white sand.

during which 48 inches of water was passed through the soil. Data resulting from the study are shown in table 6.

Reaction near neutrality seems to favor phosphorus penetration and availability under the conditions of the study. In the very acid soil and in the soil containing free carbonates, phosphorus penetration from both superphosphate and rock phosphate was greatly depressed. Both rock phosphate and superphosphate made the greatest penetration in a soil almost saturated with calcium but with no free carbonates present.

The reason for more available phosphorus at pH 7.9 than at any other reaction may be explained by the low solubility of iron and aluminum and the little free calcium at this reaction.

Vegetative tests

All data accumulated thus far seemed to indicate that rock phosphate should supply the plant with phosphorus at least as well as does superphos-

TABLE 6

Available phosphorus and pH in limed and acid treated Morrison subsoil from percolation tubes after leaching with 49 inches of distilled water

TREATMENT*	SOIL†	SURFACE‡ 40 GM.		NEXT 100 GM.		2ND 100 GM.		3RD 100 GM.		P IN TOTAL LEACHINGS (48 IN.)
	pH	p.p.m. P	pH	p.p.m. P	pH	p.p.m. P	pH	p.p.m. P	pH	mgm.
None..................	3.77	None	7.01	0.36	5.06	0.28	4.54	0.24	4.44	None
Superphosphate 400.....	3.77	1.08	4.98	2.52	4.89	1.24	4.85	0.94	4.73	None
Rock phosphate 915.....	3.77	9.50	5.56	2.66	5.13	0.88	4.57	0.76	4.47	None
Superphosphate 400.....	6.37	0.76	5.40	1.46	6.22	1.62	6.37	1.36	6.45	1.320
Rock phosphate 915.....	6.37	12.75	7.02	4.00	7.02	0.72	6.81	0.56	6.54	0.110
Superphosphate 400.....	7.90	0.53	6.88	1.88	7.97	2.02	8.19	1.96	8.19	2.620
Rock phosphate 915.....	7.90	10.70	8.10	4.20	8.21	1.30	8.25	1.34	8.25	0.265
None..................	8.32	0.15	8.06	0.44	8.28	0.44	8.31	0.40	8.31	0.095
Superphosphate 400.....	8.32	0.64	8.17	1.90	8.35	1.40	8.35	1.00	8.35	0.762
Rock phosphate 915.....	8.32	1.17	8.34	0.66	8.34	0.54	8.29	0.50	8.29	0.127

* Fertilizer added and pounds per acre.

† pH of stock soil at beginning of percolation test after treatment with lime or sulfuric acid.

‡ All fertilizer was mixed with this layer.

TABLE 7

Results of analyses of soils and young plants from phosphate fertilizer test plots on which the Pennsylvania 4-year rotation is used

PLOT NUMBER	TREATMENT*	SUM OF 4 YEARS' YIELD OF CROPS†	CROP SAMPLED	P_2O_5 IN PLANTS‡	SOIL PROPERTIES	
					Available P	pH
		lbs.		per cent	p.p.m.	
1	L, P, K, superphosphate	13,809	Wheat	0.640	20	5.81
2	L, 2P, K, rock phosphate	10,476	Wheat	0.384	93	6.08
27	L, manure, + P, superphosphate	16,589	Wheat	0.941	30	6.16
28	L, manure, + 2P, rock phosphate	14,491	Wheat	0.821	69	6.23
32	L, manure, + 4P, rock phosphate	15,602	Wheat	0.878	281	6.17
1	L, P, K, superphosphate	13,809	Hay	0.183	22	5.81
2	L, 2P, K, rock phosphate	10,476	Hay	0.184	41	6.62
27	L, manure, + P, superphosphate	16,589	Hay	0.246	15	6.30
28	L, manure, + 2P, rock phosphate	14,491	Hay	0.208	45	6.05
32	L, manure, + 4P, rock phosphate	15,602	Hay	0.203	63	7.15

* L—lime to meet Vietch lime requirement.

P—48 pounds phosphoric acid per acre applied on corn and wheat.

K—50 pounds potash per acre applied on corn and wheat.

Manure—6 tons per acre.

Superphosphate—16 per cent superphosphate applied on corn and wheat.

Rock phosphate—28 per cent brown rock phosphate.

† From data by Noll, Gardner, and Irvin (10).

‡ On oven-dry basis.

phate. Since there is abundance of evidence that rock phosphate does not usually support crop growth as well as does superphosphate, further experiments were planned in an effort to determine why the solubility and plant response tests of these phosphatic fertilizers when applied to soils do not agree.

Samples of plants from certain phosphorus treated plots were obtained, dried, ground to pass a 1-mm. sieve, and analyzed for total phosphorus. Soil samples from the same plots were tested for available phosphorus and acidity. Table 7 gives the results of these analyses. These data indicate that in general there is a tendency for young plants grown on soils fertilized with superphosphate to have a higher phosphoric acid content than do plants on rock phosphate treated soil. Also hay, which is largely clover in this case, was as high in phosphoric acid where rock phosphate was used as where superphosphate was used, when manure was not applied. This might be explained by Truog's (16) theory on the ability of plants to absorb nutrients. He believes clover plants absorb large amounts of calcium, which tends to make the rock phosphate more soluble. Data presented here, however, are not held out as proof of the theory.

In all cases available phosphorus was greater in the soils receiving rock phosphate than in those receiving superphosphate. In general the rock phosphate plots were more alkaline than the superphosphate plots. This is in entire agreement with previous data.

SUMMARY

Amounts of available phosphorus at 15 depths in the upper foot of permanent pasture plots were determined by the Truog method. The data show that biennial surface applications of superphosphate penetrate not more than 2 or 3 inches and perhaps much less in 16 years. Rock phosphate applied in the same manner penetrated more than 7 inches. Eight years after the last application of rock phosphate and three years after the last superphosphate application the rock phosphate pasture contained 188 pounds per acre of available phosphorus; and the superphosphate plot, 18 pounds per acre. Rock phosphate applications contained four times as much phosphorus as did superphosphate applications.

Laboratory percolation studies show that more phosphorus is available in rock phosphate treated soils of wide range of pH and treated with various nitrogen carriers than in the same soils treated with superphosphate. Rock phosphate penetrated more rapidly than superphosphate in an acid soil, but the reverse was true in alkaline soils. Ammonium sulfate or sodium nitrate speeded the penetration of rock phosphate more than that of superphosphate, especially in an acid soil.

The field and laboratory penetration studies all show more soluble phosphorus in rock phosphate treated soils than in superphosphate treated soils. In most field tests in which a comparison has been made of superphosphate

and rock phosphate, however, superphosphate has proved the better phosphorus fertilizer.

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THE EFFECT OF NITRATE-NITROGEN ON THE CARBOHYDRATE METABOLISM OF INOCULATED SOYBEANS[1]

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A number of investigations indicate that closely associated with the carbohydrate-nitrogen balance in legumes is the process of nitrogen fixation; the nitrogen available to the plant is dependent, at least in part, upon the fixation process, which in turn is thought to be directly associated with available carbohydrate for protein formation and for energy requirements of the bacteria in the nodule.

It has been known for many years (3) that the production of nodules may be inhibited by the presence of various inorganic nitrogen compounds, and recently (4) a number of organic nitrogen compounds have been shown to produce the same effect. A mass of data on this detrimental effect, especially by nitrates, has been accumulated without throwing much light upon the actual mechanism of either the fixation process or its inhibition. Hopkins and Fred (4) postulate as a modification of Maze's theory, that a well-balanced carbon-nitrogen ratio is maintained in plants receiving combined nitrogen so that the concentration of carbohydrate available to the bacteria is insufficient for normal growth and energy requirements. This assumption is supported by Strowd's (9) data in which a marked reduction of sugars is accompanied by the presence of nitrate in the sap. These data, however, are very meager. The purpose of the present experiments was to make a more detailed examination of the effect of nitrates on the sugar level in the sap of inoculated soybeans.

EXPERIMENTAL

Four experiments were made in which soybean seed, of the Manchu variety, was sterilized (5) and grown in 2-gallon pots in sterile, nitrogen-free pit sand. Each pot, containing 10 plants, was inoculated with an effective strain of *Rhizobium japonicum* (in the fourth experiment the plants receiving nitrate were left uninoculated). The plants were watered with distilled water and nitrogen-free Crone's solution (1). Nitrate in five or six concentrations was added to each pot three times a week for 4 weeks, so that the nitrate concentration was held at a fairly uniform level during the first 4 weeks of growth; 0

to 300 mgm. of combined nitrogen was added per week. The nitrate salts used in experiments 1 to 4 were respectively:—calcium, sodium, potassium, and ammonium.

The pH was determined on the plant sap by the quinhydrone electrode. Nitrate-nitrogen was determined on the fresh tissue by a modification of the phenol-disulfonic acid method (2). Reducing sugars were determined on the fresh tissue by 80 per cent alcoholic extraction. After evaporation of the alcohol to a small volume the sugars were determined by a modification of the Shaffer-Hartman micro method (8).

DATA AND DISCUSSION

The inoculated plants receiving no nitrate went through the usual nitrogen starvation period. At about the third week fixation commenced as evidenced by greener leaves and increased growth. The plants receiving 15 and 30 mgm. nitrogen per week had green leaves and were similar to the 0 series at the

TABLE 1

Effect of Ca(NO₃)₂ and NaNO₃ on pH of plant sap

	ROOTS				TOPS		
NO₃-N added weekly for 4 weeks	Ca(NO₃)₂ 5 weeks	Ca(NO₃)₂ 6 weeks	NaNO₃ 6½ weeks	NO₃-N added weekly for 4 weeks	Ca(NO₃)₂ 5 weeks	Ca(NO₃)₂ 6 weeks	NaNO₃ 6½ weeks
mgm.	*pH*	*pH*	*pH*	*mgm.*	*pH*	*pH*	*pH*
0	6.00	....	5.83	0	5.77	....	5.96
35	6.03	5.97	6.42	35	5.88	5.94	5.98
70	6.04	5.98	6.31	70	5.91	5.94	6.00
140	5.97	5.94	6.42	140	5.86	5.90	6.04
280	6.30	6.08	6.47	280	5.88	6.00	6.09

4½ week harvest. The plants of the 70 mgm. series had a leaf area and plant size somewhat larger than those of the 0, 15, or 30 series. The plants receiving 150 mgm. of nitrogen similarly had large leaf areas and plant size. At an addition of 300 mgm., however, the plants began to show an excess of nitrate—occasional leaf curling.

Since the same general indications were apparent in each of the four experiments, only representative cases will be given and discussed.

pH of the plant sap. When nitrate is applied to plants a certain concentration may be reached which is definitely toxic as evidenced first by leaf curling and later by a dwarfed plant. One of the factors involved is the unused cation of the salt. In the case of Ca, K, and NH₄ salts, where the plant utilizes the cation in its metabolism, the toxic effect is somewhat counteracted. With sodium nitrate, however, an accumulation of sodium is to be expected because of the inability of the plant to utilize this ion. Such a condition raises the pH of the sap to a marked degree. This is shown in table 1, which gives

a comparison of pH values at the different levels of nitrate between plants receiving sodium nitrate and those receiving calcium nitrate. There is no particularly significant rise except in the roots of plants receiving the sodium salt; at the lowest concentration of sodium nitrate added, the pH rises 0.6 higher than in inoculated plants receiving no nitrate.
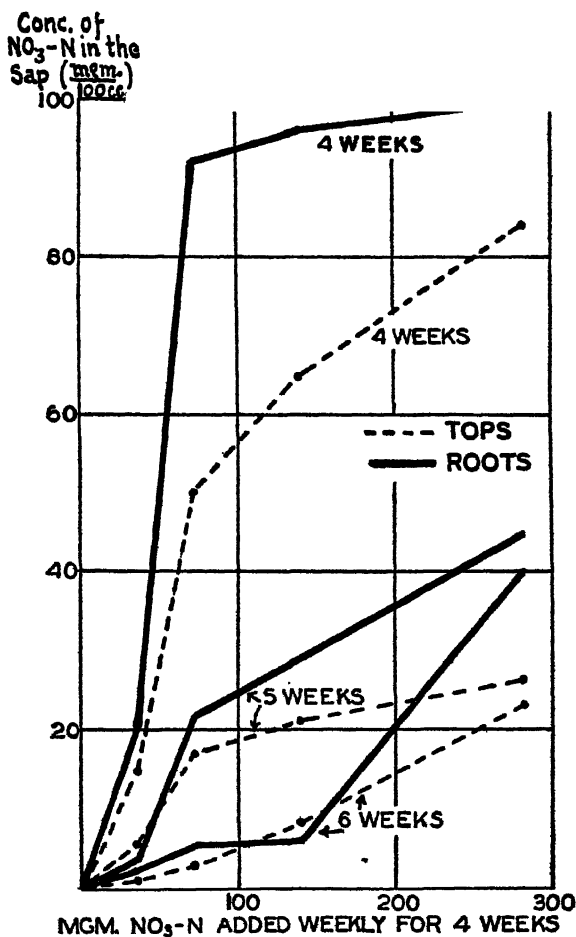
FIG. 1. COMPARISON OF NITRATE IN THE SAP TO NITRATE ADDED

Nitrate concentration in the sap. Although the concentration of nitrate in the sap is not strictly proportional to that added, as pointed out by Strowd (9), it builds up rapidly and in general approaches a direct proportion in the higher concentrations as shown in figure 1. At 4 or 5 weeks (fig. 1) the lowest concentration of nitrate added has a disporportionately low value in the sap. The nitrogen was apparently utilized more or less completely by combining with carbohydrate to form protein. In the next set of plants (at an addition

of 70 mgm. per week) the concentration of nitrate in the sap rises abruptly, apparently because of an excess over that required by the available carbohydrate. The last nitrate is added at 4 weeks, and as the stored nitrate-nitrogen is used up, all of the concentrations in the sap tend to fall to lower values at 6 weeks, and are then proportional to the nitrogen added.

Soluble sugar in the sap. The addition of nitrate considerably lowers the reducing sugar content of plant sap (fig. 2). The effect may be divided into three phases depending upon the concentration of nitrate used. These data and their interpretation are as follows:

1. A small concentration of nitrate (15 to 30 mgm.) in the substrate causes an immediate drop in the reducing sugars in the sap. This indicates that the nitrogen added in addition
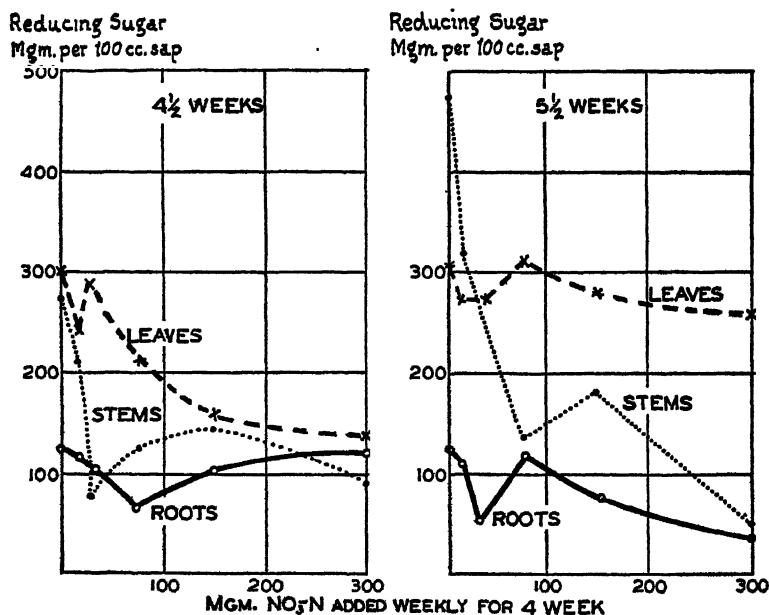
FIG. 2. EFFECT OF NITRATE ON GLUCOSE CONTENT OF PLANT SAP

to that being fixed in the nodules combines with the available sugar to form protein; the sugar level in the sap is thereby reduced and a minimum point is formed on the sugar curve.

2. As the concentration of nitrate is increased to a concentration just below that which will inhibit nodule production (70 mgm.), the size and leaf area of the plant are increased. This turns the sugar curve upward to form a maximum point. This increased photosynthetic capacity allows a partial recovery of the sugar concentration in the sap, since not enough nitrate is present for the utilization of the additional sugar formed.

3. As the nitrate concentration is still further increased, and nodule production is inhibited, the reducing sugar concentration goes down to low values at the highest concentration of nitrate tolerated by the plant. This further lowering is probably due to the increased consumption of glucose in protein formation.

This same general phenomenon, as plotted in figure 2, was also observed for sucrose. The sucrose content of the sap was approximately 50 per cent of

the soluble sugars; the sucrose graphs for the first four weeks were very similar to those for glucose.
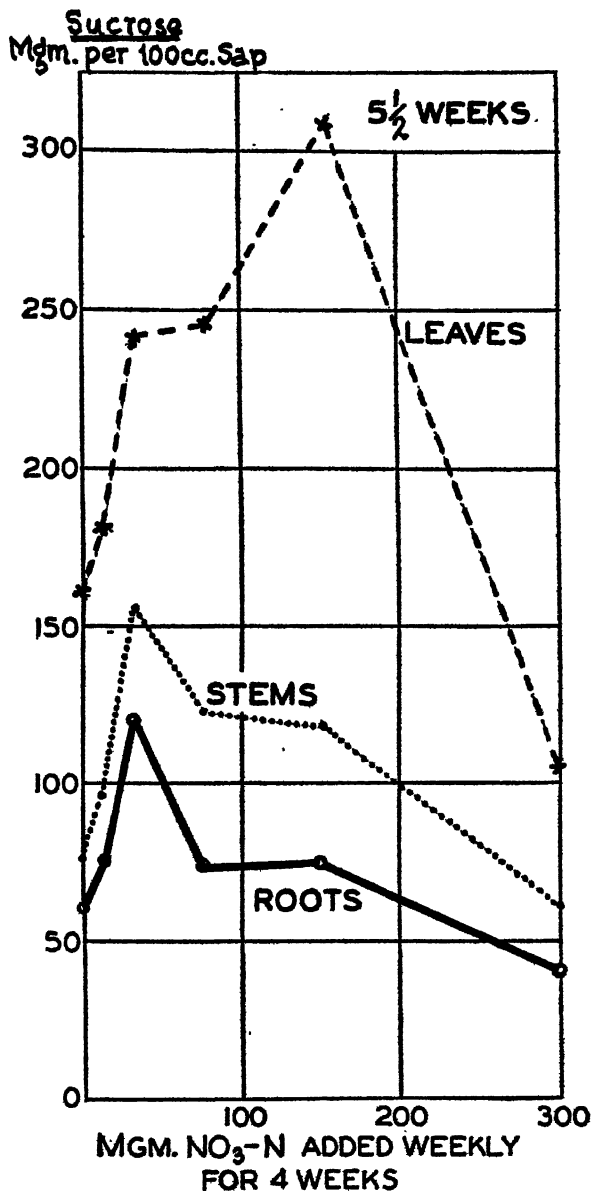
FIG. 3. SUCROSE CONTENT OF PLANT SAP AFTER NITRATE ADDITIONS HAVE CEASED

After 4 weeks the addition of nitrate was discontinued, so that the nitrogen supply for plants growing in the lower nitrate levels was soon exhausted

(fig. 1). The comparative concentrations of sugars under these conditions are shown in figure 3. Instead of a sharp decline in sugar, as in figure 2, the sugar concentration rose in the lower nitrate treatments, followed by a reduction. The change in type of curve from figure 2 to that of figure 3 was noticed in the various experiments to occur first for sucrose at approximately 5½ weeks, followed by glucose a week or more later. Factors that may have contributed to this change were at least two:

1. Since the nitrogen-hunger period in soybeans lasts until after the third week, the fixation process in plants harvested at 4 weeks was just getting underway. At 5 and 6 weeks
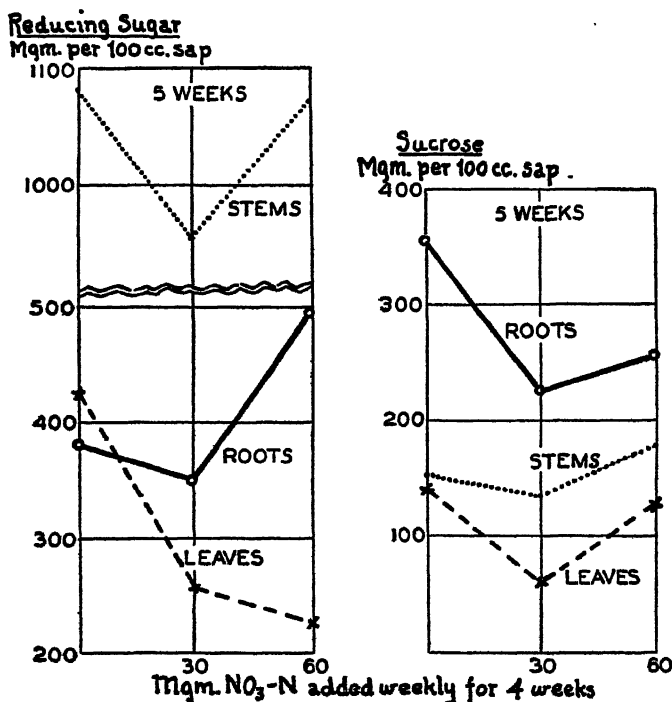
FIG. 4. EFFECT OF NITRATE ON SOLUBLE SUGAR IN SAP OF UNINOCULATED PLANTS

much larger quantities of nitrogen were being fixed, therefore the sugar level would tend to be lowered at these later harvests as more nitrogen was made available for protein formation.

2. In the plants that received low levels of nitrate, a photosynthetic stimulation, as has been noted, maintained and even raised the sugar level after the nitrate was depleted, in spite of the nitrogen supplied by fixation. In the higher nitrate applications enough nitrogen still remained to combine with the sugar formed and hold the free sugar at a lower level.

Since all plants in these experiments were inoculated, it is not clear whether the observed effects on the sugar content of the sap are due directly or indirectly to the combined nitrogen. It is possible that physiological effects of combined nitrogen on nodulation and nitrogen fixation are factors in regulat-

ing the carbohydrate level. To settle this question an experiment was made in which all plants receiving combined nitrogen (as NH_4NO_3) were uninoculated. Only the lower concentrations of combined nitrogen were used, since the occurrence of the maximum and minimum points in the curves was noted in previous experiments at concentrations less than 75 mgm. The data in figure 4 show that there is an initial reduction in sugar followed by a rise in plants which received all their nitrogen as NH_4NO_3. The results of these experiments would indicate that the effect of combined nitrogen on nodulation and nitrogen fixation is probably an indirect one, *viz.*, the presence of combined nitrogen lowers the sugar content, which affects the development of nodules. It appears that any direct physiological effect of combined nitrogen on nodulation is relegated to a secondary rôle.

Roux and Burgevin (7) have recently shown that the total quantity of nitrogen *fixed* increases with nitrate treatment within certain limits. This may well be explained by the increased carbohydrate made available through a photosynthetic stimulation by nitrate as observed in the present experiments. It has also been observed (10) that increased photosynthetic capacity increases nodulation, which is very probably a factor in increased nitrogen fixation.

SUMMARY

Data concerned with the carbohydrate metabolism of soybeans are presented which support a biochemical explanation of the effect of nitrate-nitrogen on nodule formation. In concentrations of nitrate *that do not prevent nodule formation* the addition of nitrate can effect the carbohydrate content in the following ways:—

A low nitrate concentration reduces the level of soluble sugars in the plant sap—probably through protein formation.

Intermediate concentrations of nitrate apparently stimulate photosynthesis, as evidenced by increased soluble sugars. It has been noted that this is associated with increased nodulation and nitrogen fixed (7, 10).

Higher nitrate concentrations again decrease the soluble sugar level because the abundant nitrogen source has made the photosynthetic capacity the limiting factor in protein formation. This second reduction of sugars is associated with decrease in number and size of nodules.

It appears that the carbohydrate level in the plant is correlated with nitrate-nitrogen concentration in much the same way as is nodule production and thus supports a chemical explanation of the phenomenon rather than a physiological interpretation.

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THE EFFECT OF LONG AND SHORT DAY AND SHADING ON NODULE DEVELOPMENT AND COMPOSITION OF THE SOYBEAN[1]

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The fundamental problem of the necessity of light for the subsistence of plant life has received the attention of workers in many fields of science. In studies on root nodule formation by leguminous plants, however, light has not been considered in the same detail as have other factors which determine plant growth. Scattered observations upon nodule development under poor conditions of illumination have been made, but in only a few cases have data been recorded on plant response related to nodule development.

The seasonal variation in the intensity of light and length of day on nodule formation was considered by Leonard (15). Successive plantings were made of three varieties of soybeans in sand and soil at intervals of a month throughout almost a year. On those plants grown during the winter months when the days are short and the light intensity is low the number of nodules was at a minimum. Also, during the winter the plants produced the lowest dry weight. Decrease in dry weight was greater in plants of the same series than in those grown in soil. It was also found that removal of leaves greatly decreased the number of nodules which formed. It is suggested that these results are related to the decreased photosynthetic activity of the plants.

Studies of the photoperiodic response of plants grown under artificially shortened or increased day-lengths have included a number of leguminous plants. Comparison of nodule development under short and long daily periods of illumination have been reported by several workers. Rosenfels (23) grew Wisconsin Early Black soybeans in a rich and in a poor soil, exposing half of the plants to a 7-hour day and half to full day-length (October) with artificial lighting from 4–10 p.m. The results are expressed as the percentage of plants which developed nodules. A higher percentage of the short day than of the long day plants developed nodules, and within one length of day treatment the plants in the poor soil were better provided with nodules than were those in the rich soil. Carbohydrate analyses indicated the highest carbohydrate percentages to follow the best yields of nodules.

Tincker (30) exposed cultures of *Phaseolus multiflorus* to 5-hour, 10-hour,

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[1] Contribution from the Hull Botanical Laboratory, University of Chicago.
[2] National Research Council Fellow.

and full day illumination. The plants grown in 5 and 10 hours of light possessed heavy, tuberized roots on which no nodules had formed, while plants grown in the full day did form nodules. The short day plants contained less fiber, but more crude protein and starch, than did the long day plants.

Wisconsin Early Black variety soybeans were grown by Eaton (5) in lengths of day of 3, 4, 5, 6, 8, 10 and 12 hours and full day length. The weight of nodules on these plants increased with the length of day, as did also the absolute amount of carbohydrates. In plants subjected to removal of leaves, the same relations were found to hold true.

Allam (1) attempting to determine the energy relation of nitrogen fixation in leguminous plants, gives results with peas and soybeans, a part of which were grown under short day conditions. The day length was not constant, but varied from 6 to 8 hours. Nodules on the short day plants were noticeably smaller than on the long day plants.

The application of nitrate to plant cultures is an important factor influencing nodule development, and will be dealt with in this paper. It will not be necessary to include the large number of references on the result of nitrogen fertilization on nodule development inasmuch as reviews are available (6). The papers considered here will be only those dealing with the composition of leguminous plants to which nitrates have been applied. Welton and Morris (32) grew Elton and Hamilton variety soybeans in poor, medium, and rich soils. The number of nodules decreased definitely with increasing fertility of the soil. Analyses of the lower two-thirds of the stems showed little difference between the percentage of available carbohydrates in plants from the poor and medium soils, though plants from the rich soil were lower in carbohydrates than were plants from poor or medium soil. Total carbohydrate percentages, including cellulose and lignin as well as the available carbohydrates, showed decreasing order with increasing soil fertility. The total, insoluble, and soluble nitrogen content of plant stems from the poor and rich soils were similar.

Thirteen periodic harvests were made by Rüffer (24) of Mammoth Yellow soybeans from three series: (a) nitrate, no nodules, (b) nitrate-nodule, (c) nodule. The nodules on the plants receiving nitrate were considerably smaller than those on the minus nitrogen plants. The application of nitrogen resulted in higher yields of plant material as fresh weight after the first few harvests, and as dry weight in later harvests. The total amount of nitrogen and the percentage of nitrogen in the tops and whole plant were higher in the nitrate-nodule series than in the nodule series until near the end of the experiment. Contrasted with the relation between nitrogen content in the three series is the content of sugar and starch. The nodule series was higher in percentage of sugars until the seventh harvest, and in starch until the last harvest. This work will be discussed in more detail later.

Reports of a few investigators had made it appear promising that a detailed examination of the nitrogen and carbohydrate constituents of leguminous plants growing under various conditions of light and nitrogen nutrition might

indicate some relationship between plant composition and nodule development. Also, various lines of indirect evidence appeared to suggest that such a relation did exist. Those treatments which tended to produce a high carbohydrate type of plant, as low nitrogen supply, increased CO_2 pressure, long day illumination, and bright light also are notably favorable for nodule development. Accordingly, experiments were planned to produce plants of varying carbohydrate composition from low to very high, with coincident inverse nitrogen composition.

The work of Garner and Allard (7) has shown that medium and late varieties of soybeans are definitely short day plants. Associated with the phenomenon of flowering in short day, the soybean accumulates considerable carbohydrate. Accumulation of nitrogen also occurs in such plants (18). These facts led to the selection of the soybean as the experimental plant. The following experiments will be reported here:

1. Long and short day experiments.
    Exp. 1. Plants grown under long and short photoperiods with varying levels of nitrogen nutrition.
    Exp. 2. Plants grown under long and short photoperiods without nitrogen.
2. Shading experiments.
    Exp. 3. Plants grown with and without shading with nitrate supplied.
    Exp. 4. Plants grown with and without shading without nitrogen.

METHODS

The plant used throughout the work was the Manchu variety of soybean.[3] Two- and three-gallon glazed coffee urn linings served as pots. These were filled with No. 3 quartz sand. For inoculation of the seed, one strain of *Rhizobium japonicum*, the Wisconsin strain number 504, was used. Inoculation was effected at the time of planting by suspending the bacterial growth from cultures about 2 weeks old in distilled water and soaking selected soybean seed in the bacterial suspension for about half an hour. In experiments 1 and 3 the seed was planted in beds of No. 3 quartz sand and seedlings were transplanted to the pots. In experiments 2 and 4 the seed was planted directly in the pots and selection made of the seedlings. No noticeable difference between the transplanted and directly planted seedlings was apparent.

For the first 2 weeks the plants were watered with tap water and after that time, with nutrient solutions. In experiment 1, the solution was supplied by the constant drip method of Shive and Stahl (26). In the other experiments water or nutrient solution was poured into the pots. In experiments 3 and 4, since the plants were out-of-doors, the pots were set in shallow granite-ware pans to prevent wide fluctuations in water supply. In all experiments, the pots were flushed out with about a gallon of water at weekly intervals. An attempt was made to maintain fairly constant pH conditions in each experi-

[3] The author is indebted to the A. L. Dickinson Seed Company of Chicago for the seed used.

ment. The pH in the several experiments was varied because the optimum pH for nitrate nutrition is different from that for nitrogen nutrition from nodules, as will be discussed later.

Two nutrient solutions were used. In experiment 1 and for the first crop of experiment 3 a solution used by Tiedjens and Robbins (29) was applied, and in the remaining experiments a Bryan's solution (3) was used. These were made up as minus nitrogen solutions and so used in experiments 2 and 4, whereas varying amounts of nitrate were added in experiments 1 and 3. The composition of these basic solutions expressed on the partial volume molecular basis is as follows:

Bryan's solution		Tiedjens and Robbins' solution	
K_2HPO_4	0.00143	KH_2PO_4	0.00633
$MgSO_4 \cdot 7H_2O$	0.00102	$MgSO_4 \cdot 7H_2O$	0.00237
$CaCl_2$	0.00225	$CaCl_2$	0.00146

The amounts of nitrate applied in each series will be given as each experiment is described.

In experiments 1 and 2, in which the plants received a long and a short photoperiod, the pots were placed on trucks. The "short day" plants received 7 hours of illumination, from 9 a.m. to 4 p.m. daily. For the long nights the trucks were wheeled into light-tight ventilated rooms. The temperature in these rooms was lower during the late afternoon than that in the greenhouse, but the night temperatures were nearly identical. The long days were obtained by supplementing the prevailing length of day from 5 to 9 p.m. with 1000-watt lamps. Shading in the out-of-door experiments—3 and 4—was produced by a gabled roof built over the table supporting the pots and covered with unbleached muslin. The muslin extended to the eaves of the roof, which were 4 feet above the table. The plants thus received full sunlight in the early morning and late afternoon, and were shaded through the middle of the day for a period of 5 to 7 hours.

The light transmission of the shade was determined by means of a MacBeth illuminometer, and the readings were taken with the test plate directed toward the sun. Under these conditions, the light measured under the shade was 16.9 per cent of the light outside on a bright sunny day. By the end of the experiment, the light transmission had been decreased by dust accumulation on the cloth to 11.7 per cent of full sunlight. The "sky" or diffuse light was reduced under the shade to 71.4 per cent of that outside.

Microchemical tests for starch and nitrate were made from time to time on the plants.

Plant harvests

The plants were subjected to the same light treatment on the night before harvesting as had been given throughout the experiment. Harvests were started in the morning, but in some cases the entire day was required to care for the plants in the different series.

Nodules were first picked from the roots, and the roots were severed near the sand line. The stems were picked free of leaves (including pulvini) and blossoms, and the upper inch was removed. In experiment 3, second crop, the lower stem fraction is that part from the base upward for 4 inches. Petioles were removed from the leaves and put into a heterogeneous fraction made up of stem tips, leaves of one third full size or smaller, buds, and fruit. This fraction was used for obtaining total plant weights and was not analyzed for carbohydrates. However, in order to obtain the nitrogen balance of the plants in experiments 2 and 4, nitrogen fractionations were made of the "Petiole, etc." fraction. The rachis bearing the trifoliate leaf was included with the leaf blades. The plant material was minced with a sharp knife, and that part used for carbohydrate and total nitrogen analyses was dried according to the procedure given by Link (16).

The samples for nitrogen fractionation were taken from the fresh material. In experiment 1 the tissue was extracted at once, but in the remaining experiments it was preserved by freezing and was maintained at -5 to $-10°C$. until analyzed a short time later (19).

Carbohydrate methods

The dried plant material was ground roughly in a hand drug mill, and the grinding completed in a ball mill.

Obvious steps in the analytical procedure will be omitted, and only those points given which are essential to the methods. The sugar determinations were made by a micro Shaffer-Hartmann method (27). In a large number of analyses this method has proved reliable and sufficiently accurate. It is also a rapid method. Sucrose was inverted with HCl (2).

Starch and dextrin were extracted from the sugar-free residue by boiling with water for about 5 minutes, and maintaining the solution at $80°C$. for half an hour to an hour. The solution was cooled to $38°C$. and 10 cc. of saliva added. Starch which had remained in the plant tissue during the heating and digesting treatment was removed by this procedure. The residue was always examined microscopically for the presence of starch and if none was found, the determination was continued. The maltose from the starch hydrolysis was converted to glucose by the method recommended by the Committee on Methods of Chemical Analysis of the American Society of Plant Physiologists (Willaman et al. 34).

By "total carbohydrates" is meant the sum of total sugars and starch and dextrin. The figure represents the readily available carbohydrates in the plants.

Nitrogen methods

Preparation of soluble nitrogen extract. In experiments 1 and 3, 5 or 10 gm. of the minced and well-mixed plant tissue were ground and extracted with water

(18), and the extract was filtered through a pad of rag pulp on a Büchner funnel. In experiment 2, 10- to 20-gm. samples of the plant parts were used for nitrogen fractionation, and in experiment 4, 25 to 50 gm. of nodule material and 100 gm. of the other plant parts. It had been found (4) that extraction by boiling water removed the soluble nitrogen from plant material as efficiently as did grinding. The material from experiments 2 and 4 was extracted by boiling for 10 minutes, acetic acid was added to give a concentration of about 0.02 per cent, and boiling was continued for 5 minutes more. To facilitate removal of the soluble nitrogen, the nodule material was crushed before being extracted. After boiling, the material was washed and squeezed out a number of times and the extract filtered through a pad of rag pulp. Extracts prepared in this manner do not contain coagulable nitrogen, but only the "soluble nitrogen" of the plant material.

Nitrogen fractions determined. The total nitrogen of the extract was determined by the method recommended by Ranker (22). The ammonium nitrogen and the nitrate nitrogen were determined on duplicate aliquots by the method of Sessions and Shive (25). Amide nitrogen was split off by hydrolysis (19) and the resulting ammonia determined by aeration. The total nitrogen in the humin precipitate was determined for some of the early samples, but it constituted so small a part of the total soluble nitrogen that the precipitates from the later samples were discarded. The humin filtrate was treated according to the procedure of Osborne and Harris (21) for determination of the phosphotungstic acid precipitable nitrogen. This fraction will hereafter be referred to for convenience as "basic nitrogen" though only a small portion of the fraction is made up of the diamino acids. The filtrate was used for the α-amino nitrogen determination by the usual Van Slyke method.

All results are expressed as percentage nitrogen in the form given, not, for example, as NH_3 or NO_3.

The total nitrogen was determined on the dried samples by the Kjeldahl method modified to include the nitrogen of nitrates. "Insoluble nitrogen" is obtained by subtracting the figure for soluble nitrogen from that for total nitrogen. In experiments 1 and 3, the "inorganic nitrogen" determinations were made on the dried material, and include the nitrogen of both nitrate and ammonia.

In spite of the fact that small samples were taken in experiments 1 and 3 for the estimation of total extractable nitrogen, the amount of nitrogen found in duplicate aliquots checked satisfactorily in most cases. It is possible by making analyses on a semi-micro scale to perform nitrogen fractionations on 10 to 20 gm. of material as was done in experiment 2. Difficulty because of the small size of the sample was encountered only in the determination of amino nitrogen.

Insoluble ash was determined in the dried root samples, and data given for the roots are on the insoluble ash-free basis.

Plant growth

Experiment 1 (Greenhouse). The seed was planted March 9 and the harvest made April 25, 1932. Two series were set up in both long and short day, a very low nitrogen series, and a high nitrogen series. The nitrate, KNO_3 expressed as mgm. per liter of nitrogen added to the basic Tiedjens and Robbins' solution, is as follows: low nitrogen—2 mgm. 10th to 22nd day, 5 mgm. 23rd to 29th day, and 10 mgm. thereafter; high nitrogen—20 mgm. 10th to 20th day, 40 mgm. thereafter.

The pH of the nutrient solution was 5.2. The pH of drippings from the pots was 5.2 to 5.8.

There was no symptom of the usual nitrogen-hunger period[4] in any set except the low nitrogen, long day plants. A yellowing of the leaves began in this set about 4 weeks after planting. The low nitrogen, long day plants were hard and woody, and rather pale green. The high nitrogen, long day plants were green with large leaves, and were rapidly vegetative and somewhat taller than the low nitrogen set. Both sets of long day plants possessed flower buds at the time of harvest. The short day plants were somewhat paler in color than the long day high nitrogen plants and were considerably branched, but otherwise appeared like young plants. There were, of course, numerous blossoms on both the low and high nitrogen sets.

At no time were more than traces of nitrate nitrogen found in the long day, low nitrogen plants. The long day, high nitrogen plants gave strong tests for nitrates in all parts of the plant at most times. A slight accumulation of starch was noted in the low nitrogen set on the 34th day, but this had disappeared by the 47th day.

In the short day plants, nitrate accumulation was first noted on the 24th day in the high nitrogen set, and on the 34th day in the low nitrogen set. Between the 34th and 42nd days starch accumulation in the bases of stems started in both sets. At this time, large grains were present in the pith, xylem parenchyma, and phloem in what seemed to be similar amounts in both sets.

The treatments used considerably affected the size and distribution of nodules on the roots of plants in the various sets. On the long day, low nitrogen set, the nodules were very large; many of them were near the crown of the plant, though some were distributed over the fibrous roots. Nodules on the long day, high nitrogen set were considerably smaller, and were distributed over the fibrous roots more than were those of the low nitrogen plants. Nodules on the short day plants were considerably smaller than those on the long day

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[4] Reference will be made from time to time in describing the growth of the plants to the "nitrogen-hunger period." This stage of development is that at which the nitrogen reserves of the seed have been exhausted. Fixation of nitrogen in the nodules has begun, but is not sufficient to supply the needs of the plant. Thus, if no fixed nitrogen is given the plant, nitrogen deficiency becomes evident. After a time, nitrogen fixation in the nodules proceeds more rapidly, and the plants suddenly become dark green, and produce rapid growth.

plants. The nodules of the high nitrogen plants were smaller than those of the low nitrogen ones.

Experiment 2 (Greenhouse). The seed was planted March 4, 1933, and three harvests were made. The first was of long and short day plants, on April 22; the second, of a few pots of long day plants, on April 27; and the third, of the remaining long and short day plants, on May 20. These plants were grown without the addition of fixed nitrogen, except what was obtained from the tap water. In this experiment the pH of the solution in the pots was maintained from 5.8 to 6.5. According to whether it was desired to raise or lower the pH in the pots, Bryan's nutrient solution was made up with K_2HPO_4 or KH_2PO_4.

About 4 weeks after planting, the long day plants began to show nitrogen starvation. The short day plants showed no definite symptoms of nitrogen deficiency. The first harvest was made when the plants were still in the nitrogen-hunger stage. At the second harvest (of long day plants only) nitrogen fixation by the nodules was evident from the dark green color of the plants and rapid new growth. The third harvest took place when the plants were beginning to produce fruit.

A few tests for nitrate were made, but with negative results. After 42 days, the short day plants had begun to accumulate starch in the bases of the stems. Until the end of the experiment, the masses of starch increased until the whole stem section appeared filled. No accumulation of starch was observed in the long day plants.

At the third harvest the long day plants were nearing maturity. The short day plants remained small. The stems were about 10 cm. long, and the numerous leaves which formed were very small and clustered close to the stem. Plate 1, figure 1, gives the appearance of the plants at the third harvest.

At the first harvest, the nodules on the two series of plants were similarly distributed on the roots, that is, clustered at the crown of the plant, with a few along the tap root, but those on the short day plants were considerably smaller. At the third harvest, it was evident that nodule formation had continued in the long day plants, with more nodules present along the tap root, and that the nodules had increased in size. The nodules on the short day plants apparently had increased very slightly in number, but were somewhat larger. Plate 1, figure 1, shows the difference between the two series.

Experiment 3 (Out-of-doors). The seed of the first crop was planted July 6, and the plants were harvested August 12, 1932. The second crop was planted August 10 and harvested September 22. In this experiment the plants were given considerable amounts of nitrate as KNO_3 in the first crop and as $Ca(NO_3)_2$ in the second. By "considerable" is meant an average of about 50 mgm. NO_3—N added to each pot daily after the plants were about 3 weeks old. All parts of the plant gave strong tests for nitrate. No starch was stored at the base of the stems. The pH of cultures set in pans is somewhat more difficult to control than when free drainage takes place, but the pH was maintained most of the time between 5.0 and 6.0.

The plants in the first crop suffered considerably from a nutritional disorder which produced wrinkled leaves (9). In later experiments this condition was prevented by using a nutrient solution with a higher Ca/K ratio. Since the plants responded in the same way as did those of the second crop which had no affected leaves and showed similar chemical composition to those of the second crop, the results are included.

There was no appearance of a nitrogen-hunger period in the plants of either crop.

Nodules on the shaded plants were smaller and were more scattered over the fibrous roots, rather than clustered largely around the crown as in the unshaded plants.

Experiment 4 (Out-of-doors). The seed was planted May 23, 1933, and two harvests were made, the first on July 1, and the second on July 27. A severe nitrogen-hunger stage began when the plants were about 3 weeks old. Growth appeared to stop entirely, and symptoms of some toxicity from the tap water began to be evident. High carbohydrate plants are extremely susceptible to the effect of manganese and boron, and it is possible that the toxicity was due to these elements or to others present in the tap water. A nitrate addition was given to combat this toxicity. The pH of the cultures had been maintained at 6.0 to 7.0 up to this time. The pH was then lowered to between 5.0 and 6.0, and 20 mgm. NO_3—N as $Ca(NO_3)_2$ was added to each pot. The plants responded at once to the nitrogen addition, were green for a few days, and then returned to the nitrogen-hunger stage. At this time, when the first harvest was made, there was no nitrate present in the plant, and starch had accumulated in the stems of the unshaded plants. The nitrogen deficiency of the shaded plants had been much less pronounced than that of the unshaded ones.

The second harvest was made after pods from the first blossoms had formed.

In both experiments 3 and 4 shading resulted in somewhat taller, more slender-stemmed plants with larger, greener, and possibly more numerous leaves. Nodules on the unshaded plants were clustered about the crown, with a few scattered over the root system. The shaded plants had somewhat smaller and less numerous nodules more diffusely scattered over the roots. Figure 2, plate 1, shows the appearance of the plants and roots from experiment 4 at the second harvest.

Various questions arise from the data given on the growth responses of the plants which it may be well to discuss before proceeding to the results of the chemical analyses of the plant parts.

One of these is the important question of the hydrogen-ion concentration at which the cultures should be grown. Much has been written concerning the optimum pH for nodule development on a number of host plants (6, p. 197). The majority of workers have concluded that a reaction near neutrality, of 6.0 to 7.0, is the most favorable for nodule development and yield of leguminous plants.

The importance of nearly neutral reaction was noted in the minus nitrogen

cultures, experiments 2 and 4. When the pH of the nutrient solution contained in the pot dropped below 6.0, the leaves of the plants showed chlorotic areas which again became green within a day or two if the pH was raised above 6.0. On the other hand, plants with a thoroughly healthy appearance were produced in the nitrate experiments, experiments 1 and 3, at a pH range below 6.0. Such plants produced large nodules, and nodule formation continued through the growth of the plant. Nitrate always had the effect of decreasing the size of the nodules and also of altering their distribution on the roots, as had been previously observed (11).

In comparisons of the benefit to leguminous plants of nitrogen fixed in the nodule with nitrogen from combined sources, the plant cultures of both series have been kept at the reaction which is most favorable for nodule formation, near neutrality. That a high pH is not the most favorable reaction for obtaining maximum plant yields with nitrates has been shown for a number of plants by Tiedjens and Robbins (29) and by others. On the other hand, ammonium salts produce the highest plant yields at a reaction above 7.0. Thus the nitrate series grown near neutrality are placed at a disadvantage.

Weber (31) supplied Victoria peas with nitrate in cultures in which the reaction ranged from 6.6 to 7.6 and compared the yield and total amount of nitrogen in the plants with nodule cultures given no nitrate. Although at first, presumably until the end of the nitrogen-hunger period in the nodule series, the nitrate series yielded the greater weight of plants and total amount of nitrogen in plant yield, the nodule series were later superior. Weber draws the conclusion that leguminous plants produce greater yields and contain more nitrogen when the nodule, rather than nitrate, serves as the source of nitrogen. A contrary result is usually obtained, however, when NH_4NO_3 is used as the source of nitrogen, as in the work of Allam (1). The data of Rüffer, particularly, show generally an increased yield of fresh and dry weight of tops and total amount of nitrogen in the plants grown with nitrogen supplied as NH_4NO_3 compared with those depending on nitrogen from the nodule. In the plants of the nodule series, however, the percentage of nitrogen is usually higher late in the experiments when nitrogen fixation is proceeding at a rapid rate. Even late in the experiments, the yield of plant material still continues to be higher in the NH_4NO_3 series. The plant cultures of Rüffer varied in pH from 6.7 to 7.3. Although this range is not the optimum for the utilization of nitrate, it approaches the optimum for ammonium salts. Thus the plants in such cultures are able to feed largely on ammonium nitrogen.

It thus appears that the plants of Rüffer supplied with NH_4NO_3 produced generally larger plants containing more nitrogen than did nodule plants because the plant cultures were maintained at a pH which was favorable for the utilization of the nitrogen of the ammonium ion. The NH_4NO_3 plants did not show nitrogen starvation. Weber, using $NaNO_3$ at a pH unfavorable for its utilization, obtained greater yields from nodule plants. Although sufficient nitrogen was present in the $NaNO_3$ series, the plants showed the effect of nitrogen

starvation. The plants in experiments 1 and 3 maintained at pH of 5.0 to 6.0 and supplied with nitrate showed no nitrogen deficiency.

RESULTS

Some means of expressing the extent of nodule development in the various series was desired. The number or weight of nodules per plant would not give a fair notion of the relation between nodule development and plant growth inasmuch as both large and small plants were to be compared. An index was desired which would take into consideration the vegetative growth of the plant, and which also could be expressed on a percentage basis, as are the chemical results. The index selected as being most suitable for this work was that of the weight of nodules expressed as percentage of the fresh weight of the entire plant. This figure will be referred to hereafter for convenience as "the percentage of nodules."

In the short and long day experiments, the percentage of nodules was appreciably affected by the treatments used.

Experiment 1. Plus nitrate.	*Low N*	*High N*
Short day	2.27	1.15
Long day	3.77	2.34

Experiment 2. Minus nitrate.	*1st harvest*	*3rd harvest*
Short day	1.56	2.68
Long day	5.67	5.51

Short day conditions were less favorable than long day conditions for nodule development. Rosenfels (23) reports better nodule development on soybeans grown in short than in long day. The foregoing results show that within one day length, the percentage of nodules was decreased by the high nitrogen treatment.

The effect of shading on the percentage of nodules may be seen from the following figures:

Experiment 3. Plus nitrate.	*1st crop*	*2nd crop*
Shaded	1.43	2.21
Unshaded	3.06	3.22

Experiment 4. Minus nitrate.	*1st harvest*	*2nd harvest*
Shaded	5.17	6.56
Unshaded	5.75	7.61

There is a difference in nodule development in favor of the unshaded plants, though it may be seen that the difference between shaded and unshaded plants is smaller when no nitrate is given.

The analytical data for the experiments in which the plants received long and short photoperiods are given in figures 1 and 2, and table 1.

Figure 1 shows the results obtained from the plants of experiment 1 in which the plants received long and short photoperiods and very low and high nitrogen nutrient. The stems of the long day plants were higher in percentage moisture. Very great accumulation of carbohydrates and nitrogen had occurred in the short day plants. Total, total extractable, and inorganic nitrogen are definitely higher in short day than in long day plants. The greater nitrate

TABLE 1

Nitrogen composition, wet basis, of soybeans grown in long and short day, minus nitrogen—Experiment 2

Plants of first harvest 49 days old, of third harvest 77 days old

		AMMO-NIUM N	NITRATE N	AMIDE N	α-AMINO N	BASIC N	SOLU-BLE N	INSOLU-BLE N	TOTAL N
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Roots:									
1st harvest	Short day......	.0032	.0038	.0029	.0107	.....	.0342	.0898	0.124
	Long day......	.0040	.0004	.0026	.0007	.....	.0238	.1132	0.137
3rd harvest	Short day......	.0037	.0005	.0037	None	.0224	.0213	.0817	0.103
	Long day......	.0034	.0005	.0042	None	.0177	.0395	.1185	0.158
Stems:									
1st harvest	Short day......	.0077	.0103	.0077	.0274	.....	.4580	.2080	0.666
	Long day......	.0039	.0017	.0038	.0073	.....	.0584	.1176	0.176
3rd harvest	Short day......	.0110	.0022	.0742	.0517	.0938	.4170	.2070	0.624
	Long day......	.0017	.0015	.0164	.0144	.0216	.0995	.1125	0.212
Leaves:									
1st harvest	Short day......	.0040	.0015	.0092	.0023	.....	.1983	.7157	0.914
	Long day......	.0026	.0005	.0042	.0219	.....	.1275	.6365	0.764
3rd harvest	Short day......	.0046	.0004	.0108	.0353	.0600	.1550	.7120	0.867
	Long day......	.0022	.0007	.0127	.0170	.0449	.1290	.6550	0.784
Petioles, etc.:									
3rd harvest	Short day......	.0122	.0007	.0341	.0383	.0534	.2370	.3170	0.554
	Long day......	.0039	.0002	.0096	.0118	.0327	.0870	.2150	0.302
Nodules:									
1st harvest	Long day......	.0103	None	.0086	.0314	.....	.2935	.6635	0.957
3rd harvest	Short day......	.0258	None	.0497	.0410	.2660	.5200	.7120	1.232
	Long day......	.0137	.0047	.0274	.0531	.1801	.4445	.5735	1.018

additions in the high nitrogen series are seen to have resulted in a decrease in carbohydrate and an increase in nitrogen percentages in the long day plants. In the short day plants, the accumulation of nitrogen was somewhat increased by the higher nitrate level in the nutrient.

Soybean plants receiving a short photoperiod and given no fixed nitrogen accumulate large amounts of carbohydrates (fig. 2, exp. 2). Although the carbohydrate percentages was very high at the time of the first harvest, the total carbohydrates in the stems of the short day plants had more than doubled

at the time of the third harvest.   At both harvests, the roots of the long day plants contained higher percentages of carbohydrates than did those of short day plants.

The nodules of the long day plants contain high percentages of carbohydrates in relation to the other parts of the plant.   The percentages of carbohydrates decreased from the first to the third harvest.

Table 1 presents the nitrogen results from experiment 2.   In the roots, total and insoluble nitrogen are higher in the long day plants at both the first and third harvests.
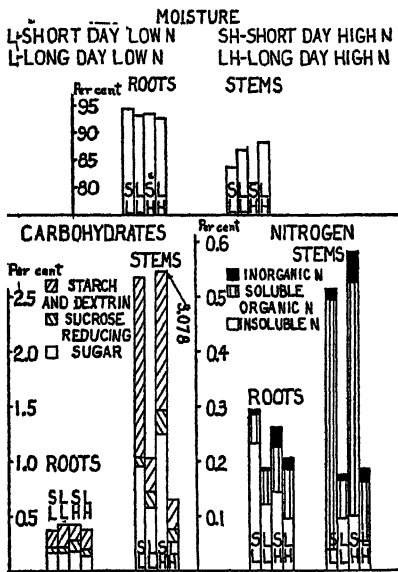
FIG. 1
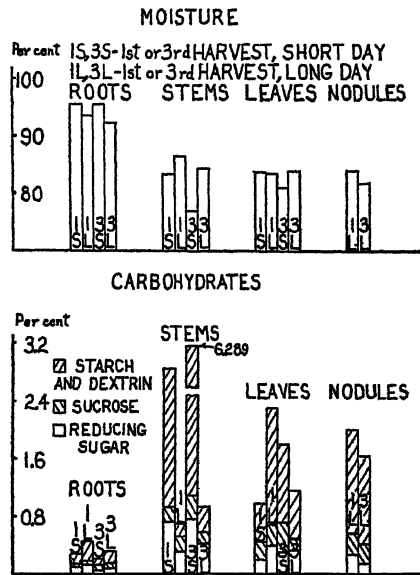
FIG. 2

FIG. 1. EXPERIMENT 1.   CARBOHYDRATE AND NITROGEN COMPOSITION, WET BASIS, OF SOYBEANS GROWN IN LONG AND SHORT DAY.   PLUS NITRATE.   PLANTS 47 DAYS OLD

FIG. 2. EXPERIMENT 2.   CARBOHYDRATE COMPOSITION, WET BASIS, OF SOYBEANS GROWN IN LONG AND SHORT DAY.   MINUS NITROGEN.   PLANTS OF FIRST HARVEST 49 DAYS OLD, OF THIRD HARVEST 77 DAYS OLD

The stems of the short day plants were higher in all forms of nitrogen at both harvests.   The leaves of the short day plants were higher at both harvests in ammonium, soluble, insoluble, and total nitrogen.   The petioles of the short day plants were higher in all forms of nitrogen than the corresponding parts of long day plants.   Nodules from the short day plants contained a higher percentage of all forms of nitrogen except nitrate and α-amino nitrogen.   If these results are computed as percentages of the total nitrogen, however, the differences between those for the short and the long day plants are not significant.

A comparison of the changes which occurred in the plants from the first to

the third harvest, will show that in the short day plants there was a percentage increase in the simpler forms of nitrogen: ammonium, amide, and α-amino. The soluble and total nitrogen percentages decreased. In order to determine whether this decrease in the soluble and total nitrogen had been due to a break-down to simpler compounds, as was indicated by the increased percentage of simpler forms of nitrogen, the various nitrogen fractions were computed as percentages of the soluble nitrogen. It was found that the amide and α-amino nitrogen made up a higher percentage of the soluble nitrogen at the time of the third harvest than at the first. Thus it appears that as short day plants grow older, the nitrogen constituents actually become more simple rather than being built into more complex forms. It is of interest that about two-thirds of the total nitrogen in the stems is in the soluble form.

In the long day plants, while the amide and α-amino nitrogen increased in percentage from the first to the third harvest, the soluble and total nitrogen also increased. The difference, it will be noted, between this result and that of the short day plants is the increase in soluble and total nitrogen. When nitrogen is applied to low nitrogen plants, the changes which take place in the nitrogen composition of the plants are similar to those seen in the long day plants (20). In this case, the plants received their nitrogen from the nodules.

The stems of the short day plants, and nodules of both long and short day plants at the third harvest were analyzed for α-amino nitrogen before and after the phosphotungstic acid precipitation. The short day stems apparently contain none of the basic amino acids, whereas about half of the amino nitrogen in the nodules is present in that form.

As has been indicated, the second harvest did not include any short day plants. Five days after the first harvest, the long day plants showed definite evidence of nitrogen fixation by the nodules. It was thought that a nitrogen fractionation performed at this time might reveal a change in the composition from that existing at the time of the first harvest. Several pots of plants were harvested, and the plants analyzed. Differences were found only in the total, insoluble, and soluble nitrogen content. All forms showed a considerable increase at the time of the second harvest.

The total amount of nitrogen in the plants at the three harvests was computed. The following figures give the milligrams of nitrogen in 10 plants.

	1st harvest	2nd harvest	3rd harvest
Short day	90.8	.....	179.8
Long day	248.8	406.3	867.6

Nitrogen in 10 seeds—83 mgm.

It is noteworthy that a very considerable amount of nitrogen had been fixed by the long day plants at the time of the first harvest, though, to all appearances, the plants were suffering greatly from lack of nitrogen. Weber (31) has obtained similar results. During the 5 days between the first and second harvests, over 150 mgm. of nitrogen were fixed by 10 plants. At the time of

the first harvest, the short day plants had fixed hardly any nitrogen, but had made a gain of about 90 mgm. at the third harvest.

The changes in nitrogen distribution in the nodules of the long day plants at the three harvests are presented in table 2. The total and insoluble nitrogen reached a maximum percentage at the time of the second harvest. Expressed as percentages of the total nitrogen the insoluble nitrogen decreased after the first harvest. The break-down of the complex nitrogen compounds in the nodule to simpler forms began soon after the first harvest. As the insoluble nitrogen decreased, the soluble nitrogen increased.

The results from the shading experiments are given in figures 3 and 4 and table 3. When nitrate is supplied to the plants, the carbohydrate percentages are low (fig. 3, exp. 3). Generally, all parts of the unshaded plants contained somewhat higher percentages of sugars and total carbohydrates than did the shaded ones. The nodules on either type of plant were higher in total carbohydrates than any other plant parts analyzed.

Inorganic, total extractable, and total nitrogen percentages were generally higher in the shaded plants than in the unshaded ones.

In the minus nitrogen experiment (fig. 4, exp. 4), the carbohydrate percentages were generally higher in unshaded plants at both the first and second harvest. Both shaded and unshaded plants showed a general decrease in starch and dextrin and total carbohydrates from the first to the second harvest. The decrease in carbohydrates in the nodules is of especial interest. The nitrogen data are presented in table 3. The nitrogen percentages in the stems, leaves, and petioles were higher in the shaded plants at both harvests. For the nodules, the results are irregular.

During the period between the first and second harvests, there was, in general, an increase in the percentage of all forms of nitrogen in both shaded and unshaded plants. The greatest increases took place in amide and α-amino, basic and soluble nitrogen. When the nitrogen results were calculated as percentages of soluble nitrogen, the change in proportion of the various nitrogen constituents during the interval between the first and second harvests were insignificant in any but a few plant fractions. At the second harvest, amide nitrogen made up a greater part of the soluble nitrogen in the stems than at the first. In the nodules, the basic nitrogen was relatively higher at the second harvest, while in the rest of the plant this fraction had decreased. If the results for basic, soluble, and insoluble nitrogen in the nodules are computed as percentages of the total nitrogen, it will be found that the basic and soluble nitrogen have increased. At the same time, there had been a decrease in the insoluble nitrogen. It is apparent that a proteolytic process was under way in the nodules, and the nitrogen made available was presumably being used for seed production (see table 2). Nodule material examined from time to time had indicated that decomposition at the center of the nodules was taking place. In other parts of the plants, the soluble nitrogen increased, and the insoluble nitrogen decreased somewhat in relation to the total nitrogen. These

TABLE 2

Nitrogen fractions in long day nodules—Experiment 2

Expressed as per cent, wet basis, and as per cent total nitrogen

	1ST HARVEST, 49 DAYS		2ND HARVEST, 54 DAYS		3RD HARVEST, 77 DAYS	
	Wet basis	Total N	Wet basis	Total N	Wet basis	Total N
Amide N	0.0086	1.0	0.0122	1.0	0.0274	2.7
α-amino N	0.0314	3.3	0.0393	3.3	0.0531	5.2
Soluble N	0.2935	30.7	0.3855	32.3	0.4445	43.7
Insoluble N	0.6635	69.3	0.8065	67.7	0.5735	56.3
Total N	0.9570	100.0	1.1920	100.0	1.0180	100.0

TABLE 3

Nitrogen composition, wet basis, of shaded and unshaded soybeans, minus nitrogen—Experiment 4

Plants of first harvest 39 days old, of second harvest 77 days old

		AMMO-NIUM N	NITRATE N	AMIDE N	α-AMINO N	BASIC N	SOLU-BLE N	INSOLU-BLE N	TOTAL N
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Roots:									
1st harvest	Shaded	.0021	.0003	.0032	.0076	.0085	.0197	.0863	0.106
	Unshaded	.0027	.0006	.0042	.0095	.0102	.0223	.0947	0.117
2nd harvest	Shaded	.0033	.0012	.0073	.0301	.0140	.0462	.1368	0.183
	Unshaded	.0026	.0006	.0052	.0119	.0096	.0324	.1136	0.146
Stems:									
1st harvest	Shaded	.0023	.0004	.0066	.0115	.0206	.0490	.1360	0.185
	Unshaded	.0016	.0006	.0048	.0130	.0163	.0410	.1270	0.168
2nd harvest	Shaded	.0071	.0020	.0259	.0329	.0297	.1212	.2298	0.351
	Unshaded	.0062	.0010	.0214	.0238	.0266	.1005	.2095	0.310
Leaves:									
1st harvest	Shaded	.0041	.0014	.0084	.0244	.0487	.0985	.5125	0.611
	Unshaded	.0031	.0006	.0065	.0222	.0400	.0860	.4170	0.503
2nd harvest	Shaded	.0065	.0019	.0197	.0418	.0733	.1820	.9410	1.123
	Unshaded	.0044	.0016	.0110	.0395	.0770	.1640	.8940	1.058
Petioles, etc.:									
1st harvest	Shaded	.0028	.0007	.0065	.0161	.0252	.0610	.2190	0.280
	Unshaded	.0021	.0012	.0060	.0177	.0254	.0547	.1883	0.243
2nd harvest	Shaded	.0077	.0029	.0233	.0332	.0461	.1461	.2919	0.438
	Unshaded	.0063	.0013	.0191	.0287	.0466	.1245	.2645	0.389
Nodules:									
1st harvest	Shaded	.0132	.0001	.0326	.0422	.1462	.2980	.5720	0.870
	Unshaded	.0172	.0001	.0284	.0426	.1030	.2310	.4650	0.696
2nd harvest	Shaded	.0191	.0042	.0580	.0872	.2954	.5560	.5230	1.079
	Unshaded	.0170	.0044	.0818	.0840	.2690	.5010	.5810	1.082

changes were relatively small compared to those taking place in the nodules. The number of milligrams of nitrogen in 10 plants was as follows:

	1st harvest	*2nd harvest*
Shaded..	322.2	2,090
Unshaded..	261.9	1,660

Nitrogen in 10 seeds—83 mgm.

Thus, the amount of nitrogen fixed by the shaded plants considerably exceeded that fixed by plants in full sunlight in spite of the fact that the nodules on the
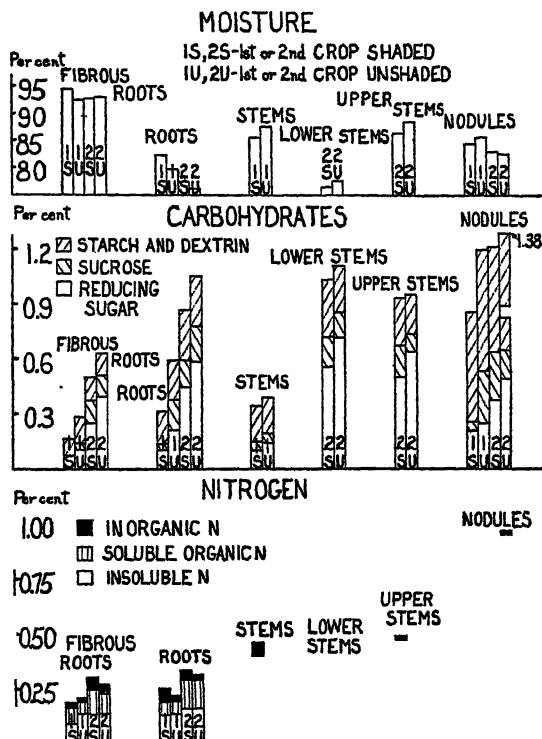
Fig. 3. Experiment 3. Carbohydrate and Nitrogen Composition, Wet Basis, of Shaded and Unshaded Soybeans. Plus Nitrate. Plants of First Crop 37 Days Old, of Second Crop 43 Days Old

latter made up a greater part of the plant weight. As was described in the section on methods, the shaded plants received direct sunlight in early morning and late afternoon. They were thus shaded during the hottest part of the day. Garner and Allard (8) have found that darkening during the middle of the day had little effect on flowering. Photosynthetic activity appears to be less in plants of temperate and some of southern origin during the hours near noon (17, 12, 13). Possibly the method of shading produced a light intensity more favorable for photosynthesis than that of full sunlight. It is certain that

fixed as ammonia or nitrate, combination of the nitrogen with carbohydrates must take place if the nitrogen is to become available to the plant. Or if nitrogen is fixed as an organic compound, carbohydrate would serve as the source of carbon.

SUMMARY

There is, generally, in the four experiments a higher percentage of sugars and starch in the roots of the plants which yielded the larger percentage of nodules. The only exception is the short day, high nitrogen set in experiment 1. Even in experiment 2, where the short day stems accumulated large amounts of sugars and starch, the roots of the long day plants contained a higher percentage of carbohydrates.

A number of lines of indirect evidence, particularly the fact that those treatments which decrease photosynthesis or reduce the carbohydrate supply of the plant are unfavorable for nodule development, had led the writer to interpret previous results (10, 11) as indicating that the carbohydrate supply of the plant was a determining factor in nodule development. The present work suggests that, with the exception of short day-length treatment, those treatments favoring nodule development generally produce plants high in carbohydrates. Of two series in long day; whether shaded or given low or high nitrate treatment, the series developing the higher percentage of nodules in relation to the whole plant weight was also usually higher in carbohydrates. The roots were more consistent in this relation than any other plant part.

The nitrogen data permit a more general statement than do the carbohydrate data. Comparing two treatments, the one which resulted in the higher soluble nitrogen percentage in all plant parts produced the lower percentage of nodules in relation to the whole plant weight. The percentages of amide and α-amino nitrogen, as well as total nitrogen in most instances, varied with the soluble nitrogen.

That those treatments favoring nodule development may not be most favorable for the highest fixation of nitrogen is shown by the results of experiment 4. Although the unshaded plants exceeded the shaded ones at both harvests in percentage nodules in relation to the whole plant weight, the shaded plants fixed considerably more nitrogen. No data are at hand which will explain this result.

CONCLUSIONS

Soybean plants were grown in long and short days, about 16 and 7 hours respectively, with a high and low nitrate series in one experiment, and minus nitrate in another. Shaded and full light experiments, plus and minus nitrate, were performed out-of-doors. Nodule formation was observed, and the plants were analyzed for carbohydrate and nitrogen fractions.

The short day-length plants accumulated much starch and were also high in nitrogen, in both the plus and minus nitrate experiments. In the minus nitrate experiment, carbohydrate accumulation increased in the short day-

length plants as the plants became older. The nitrogen was present in more simple compounds at the second harvest than at the first.

The plants receiving long day treatment were lower in all forms of nitrogen and in carbohydrates, particularly in the stems, than the short day-length plants. In the minus nitrate experiment, a first harvest was made at the time of the nitrogen-hunger stage, and another when the plants were in bloom. The carbohydrate percentages of roots, leaves, and nodules decreased, and all forms of nitrogen greatly increased in all plant parts as the plants grew older.

The shaded plants in both the plus and minus nitrate experiments were generally lower than the unshaded ones in carbohydrates and higher in all forms of nitrogen. In the minus nitrate experiment, both shaded and unshaded plants decreased in percentage carbohydrates from the first harvest, made during the nitrogen-hunger stage, to the second, made when the plants were producing fruit. All forms of nitrogen increased in both series from the first to the second harvest.

The weight of nodules expressed as percentage of the whole plant weight was decreased by high nitrate, by short day, and by shading. In general, those treatments which result in the accumulation of soluble nitrogen do not favor nodule development. Except in short day plants, those conditions which produce high carbohydrate plants are favorable to nodule development.

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PLATE 1

PLATE 1

Fig. 1. Tops and roots of plants from Experiment 2 at time of third harvest, plants 77 days old.   Long day plants, left; short day plants, right.

Fig. 2. Tops and roots of plants from Experiment 4 at time of second harvest, plants 77 days old.   Tops, unshaded plants, left; shaded plants, right.   Roots, shaded plants, left; unshaded plants, right.

E. W. HOPKINS
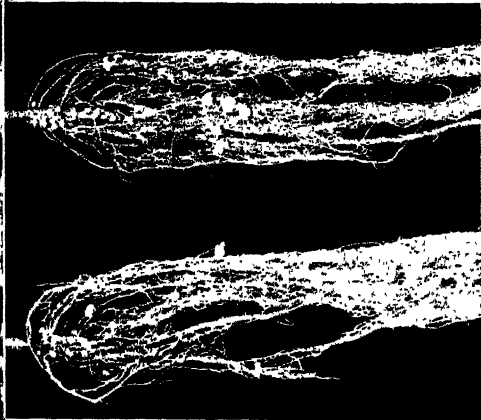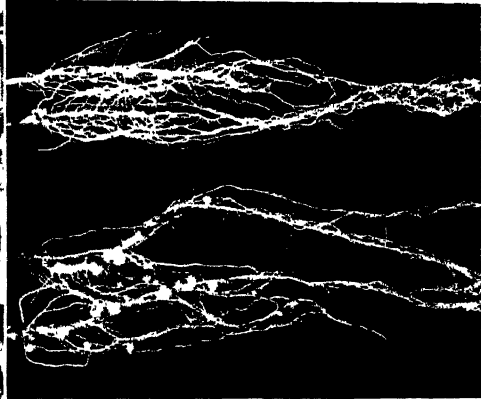
ORGANIZATION OF THE AMERICAN SECTION OF THE INTERNATIONAL SOCIETY OF SOIL SCIENCE

At a special meeting held directly following the banquet of the American Soil Survey Association on the evening of November 22, 1934, an American Section of the International Society of Soil Science was organized. The brief Constitution appended was unanimously adopted, and the following officers were elected: Dr. W. P. Kelley, of California, president; Dr. G. W. Conrey, of Ohio, vice-president; and Dr. A. G. McCall, of Washington, D. C., secretary-treasurer.

Dr. S. A. Waksman, of New Brunswick, New Jersey, and Dr. M. F. Morgan, of New Haven, Connecticut, were appointed a committee on arrangements for those planning to attend the International Congress of Soil Science to be held at Oxford, England, July 30 to August 7.

Members of the International Society of Soil Science are urged to affiliate with the American Section by sending their applications and dues to the Secretary-Treasurer, Dr. A. G. McCall, Bureau of Chemistry and Soils, Washington, D. C.

CONSTITUTION FOR THE AMERICAN SECTION, INTERNATIONAL SOCIETY OF SOIL SCIENCE

Adopted November 22, 1934, at Washington, D. C.

1. The *American Section of the International Society of Soil Science* shall be composed of those members in good standing in the International Society of Soil Science.

2. *Purpose:* The purpose of the Section will be to serve as a medium of expression for the American Members of the International Society, and as an organization through which these members can transact necessary business.

3. *Officers:* The officers shall be a President, Vice-President and a Secretary-Treasurer. They shall be elected at the annual meeting and shall serve for terms of one year, or until their successors are elected and assume office. The officers shall constitute the Executive Committee.

4. *Duties:* The duties of the officers shall be those that usually pertain to the respective offices. The duties of the Executive Committee shall be to determine policies and programs for the Section, and present their findings at the annual meetings for ratification or rejection. Between meetings of the Society, the Executive Committee shall act for the Society in matters of business.

5. *Meetings:* A business meeting shall be held annually, at such time and place as may be designated by the Section; or in case of no decision by the Section, on call of the Executive Committee.

6. *Dues:* Dues of the International Society of Soil Science are set by that body and are payable to the officers designated by that body. Dues in the American Section of the International Society shall be fifty cents per year. Annual dues may be waived or omitted for any year by decision of the Executive Committee if, in their judgment, the treasury has sufficient funds to carry on the functions of the Society without payment of dues.

THIRD INTERNATIONAL CONGRESS OF SOIL SCIENCE

The Third International Congress of Soil Science will be held in Oxford, July 30–August 7, under the presidency of Sir John Russell, Director of the Rothamsted Experimental Station, England. The two previous congresses of the series, held in Washington in 1927 and in Leningrad and Moscow in 1930, were notable for the exceptionally international character of the personnel and of the discussions. The forthcoming Congress will meet as a whole in six plenary sessions, at which a general survey of recent advances in every branch of soil science will be made, and it will also work in sections of commissions dealing specifically with (1) soil physics, (2) soil chemistry, (3) soil microbiology, (4) soil fertility, (5) soil classification, and (6) soil technology. Three subcommissions will discuss problems relating to alkali, forest, and peat soils. A 16-day excursion round Great Britain, leaving Oxford immediately after the Congress and terminating in Cambridge on August 23, has also been arranged for the benefit of members wishing to obtain first-hand knowledge of British agriculture and soils.

Every member of the Congress will receive a copy of the official transactions, including the full text of papers read at the plenary sessions, and detailed reports of the discussions at the Commission sessions. The cost of the transactions will be included in the Congress fee ($10), payment of which will also entitle members to attend all meetings, receptions, etc., held in connection with the Congress. College accommodation during the Congress can be reserved through the Organizing Committee. Those who are planning to take part in the excursion must deposit a registration fee of $10 before June 30. Intimation of attendance at the Congress should be sent as soon as possible to the Secretary of the Organizing Committee, Mr. G. V. Jacks, Imperial Bureau of Soil Science, Harpenden, England, from whom all further information may be obtained.

The Committee on Arrangements has determined that the approximate cost, for American delegates and those accompanying them, of the Congress and excursion, including Tourist transportation from New York and return to New York, will be about $380, with a reduction to members of the International Society (about $20). The committee recommends the following accommodations:

French Liner, *Ile de France,* leaving New York on July 20 and arriving at Plymouth on July 26, thus allowing three days to be spent in London before the Congress. Return on the *Champlain,* which leaves Southampton on

August 24.   However, the members have the privilege of returning on other steamers (*Champlain*, August 7[1]; *Normandie*, August 14[2]; *Ile de France*, August 21[2]; *Normandie*, August 28[2]; *Lafayette*, September 4[1]; *Ile de France*, September 11[2]) from either Southampton or Havre.   The U. S. Revenue Tax of $5 must be added to the above quotation.   It may be noted that railroad transportation from Plymouth to London as well as from London to Southampton will be provided.   Members desiring to continue to the Botanical Congress in Amsterdam (September 2 to September 7) may do so without additional transportation expense, railroad tickets being provided from London to Amsterdam, from Amsterdam to Paris, and from Paris to Havre.

The American Shipper, of the U. S. Lines leaves New York on July 20 and arrives at Liverpool on July 29.   Accommodation Tourist Class on this steamer will be about $40 less than the above quotation for the round trip, but no transportation from and to Liverpool will be provided.   One may return from London on the *American Merchant* leaving August 30.

Everyone contemplating going from the United States to the Congress is urged to write immediately to one of the members of the committee.   It is understood that the above quotation will include only the Congress, excursion, and actual transportation and will not include board and lodging and incidental expenses en route to the Congress from Plymouth and after the excursion. It is also understood that the quotation applies only if at least 25 members travel in one group going from New York.   The committee will make arrangements for reservations in line with the above plans.

[1] Slight reduction in above rate.
[2] Slight addition to above rate.

STUDIES ON PROTEIN SYNTHESIS BY THE GENUS AZOTOBACTER

ROBERT A. GREENE[1]

University of Arizona[2]

The biological fixation of atmospheric nitrogen has been the subject of many physiological and biochemical studies, particularly with reference to the metabolism of the nitrogen fixing bacteria. Several theories have been advanced to explain the mechanism of the process of nitrogen fixation, the most plausible of which is that ammonia is the first product of fixation.

The purpose of the present investigation was to determine the products of metabolism of Azotobacter, to compare the biochemical activities of the various species that comprise the genus, and to attempt to explain the mechanism of protein synthesis by these organisms.

EXPERIMENTAL

Four species of the genus Azotobacter were used in this study: *A. chroococcum* Beijerinck, *A. vinelandii* Lipman, *A. agilis* Beijerinck, and *A. beijerinckii* Lipman. Although Bergey (3) includes two additional species in the genus (*A. woodstownii* Lipman and *A. vitreus* Löhnis and Westermann), there is some question whether these species are true Azotobacter. The four species which were used have been the subject of several studies by the author, and have been carefully checked in order to insure that they were typical species.

After a survey of the literature in search of methods which might be used to collect the products of growth for analysis, and after many preliminary tests, the following scheme was adopted: Twenty-four hours prior to the inoculation of plates, the stock cultures were transferred to slants of nitrogen-free mannite agar and incubated for twenty-four hours. At the end of that time, stained preparations were made from each culture and were examined microscopically in order to check the purity of each culture. Contaminating organisms were never detected. Sterile water blanks were then inoculated with each of the cultures used. Several hours prior to this, large plates (200 mm.)

327

of the nitrogen-free agar had been poured and allowed to solidify. The medium had the following composition (8):

Mannitol..	10.0 gm.
Dipotassium phosphate (K$_2$HPO$_4$)..............................	0.5
Magnesium sulfate (MgSO$_4$·7 H$_2$O)..............................	0.2
Sodium chloride (NaCl).......................................	0.2
Manganese sulfate (MnSO$_4$·4 H$_2$O).............................	Trace
Ferric chloride (FeCl$_3$·6 H$_2$O).................................	Trace
Agar..	15.0
Distilled water..	1,000.0 cc.

When the medium had solidified, 1 cc. of the water suspension was added to the plate, which was then rotated so as to give an even distribution of the suspension upon the surface of the medium. In the latter part of the experiment, large Pyrex trays fitted with copper covers were used in place of the 200-mm. dishes (11). All glassware and media were carefully sterilized, and every care was taken to prevent contamination. The reagents used during the course of the experiment had been previously tested for absence of nitrogen. In each series of plates one culture dish was inoculated with 1 cc. of sterile water to serve as a check. In no case did any colonies develop on these control plates.

The plates were incubated at 30°C., for 4 days. This period was selected because Halversen (12) has shown that at that time 96 per cent of the total nitrogen is in the form of protein, and it is also recognized that older cultures may autolyze, liberating ammonia; also the culture of *A. chroococcum* was an active pigment former, but this pigment did not develop until after the fourth day. Rippel and Ludwig (21) have claimed that the black pigment of this species is due to the conversion of tyrosine to melanin. Consequently, the incubation period was set so that this species might be harvested prior to pigment formation.

At the end of the incubation period, the growth was scraped from the surface of the medium with a glass slide, care being taken not to remove any of the agar. Since the bacterial mass contained from 85 to 95 per cent of water, dehydration was necessary. The investigations of Omeliansky and Sieber (18) had already shown that desiccation in vacuo was not satisfactory; therefore, the bacterial mass was transferred to jars of anhydrous acetone and allowed to stand several days at room temperature, the acetone being changed at frequent intervals. When a sufficient quantity of material had been obtained, the acetone was removed by filtration through hardened filter paper, and the material was air-dried. The residue was then ground in an agate mortar, extracted and dried as before, and finally ground in a ball mill. This method of dehydration had been used successfully by Wilkerson and Gortner (25) in a biochemical study of the pig embryo. Acetone does not denature proteins and does not dissolve fats to any great extent, but does have the disadvantage, particularly when hot, of dissolving phospho-proteins, phospholipids, and similar substances.

The material, which had been obtained in the manner already described, was analyzed. A gross chemical analysis was made according to the methods of the Association of Official Agricultural Chemists (2); a proximate analysis was made, using the method of Waksman and Stevens (24); and finally a protein analysis by Cavett's micromodification (7) of Van Slyke's method. The

TABLE 1

Gross chemical composition of Azotobacter

	A. AGILIS	A. VINELANDII	A. BEIJERINCKII	A. CHROOCOCCUM
	per cent	*per cent*	*per cent*	*per cent*
Moisture.............................	8.55	9.06	5.61	5.41
Ash.................................	7.55	7.06	4.05	4.00
Protein (N × 6.25)...................	61.19	56.25	24.68	25.00
Fat.................................	0.62	1.96	0.94	3.25
Carbohydrates (by difference).........	22.09	25.67	64.72	62.34
Total............................	100.00	100.00	100.00	100.00

TABLE 2

The proximate analysis of Azotobacter

(Moisture-free basis)

	A. AGILIS	A. VINELANDII	A. BEIJERINCKII	A. CHROOCOCCUM
	per cent	*per cent*	*per cent*	*per cent*
Ether-soluble organic matter..............	0.67	2.14	0.99	3.43
Alcohol-soluble organic matter............	1.68	1.26	1.44	1.67
Cold-water-soluble organic matter.........	8.60	9.54	14.41	10.65
Hot-water-soluble organic matter..........	15.83	19.03	18.89	14.64
Hemicelluloses..........................	4.60	4.62	0.13	0.13
Celluloses..............................	0.12	0.12	0.13	0.13
Lignin-like material.....................	3.65	3.72	33.18	33.01
Crude protein...........................	50.98	47.22	15.40	17.98
Ash....................................	8.23	7.70	4.29	4.24
Recovery..............................	94.36	95.35	88.86	85.88

results, which appear in tables 1 to 4, are averages of closely agreeing duplicate determinations.

DISCUSSION

The results of the gross chemical analysis (table 1) show that *A. agilis* and *A. vinelandii* contain larger amounts of protein and ash and smaller amounts of carbohydrates than *A. beijerinckii* or *A. chroococcum*.

The proximate analysis (table 2) shows that the four species may be divided into groups: (I) *A. agilis* and *A. vinelandii*; and (II) *A. beijerinckii* and *A.*

chroococcum. The former group is characterized by larger amounts of hemi-celluloses, crude protein, and ash; while the latter group has a greater content

TABLE 3

The distribution of nitrogen in the acid hydrolysate of Azotobacter

(Results expressed as percentage of total nitrogen, not corrected for the solubility of the bases)

	A. AGILIS	A. VINELANDII	A. BEIJERINCKII	A. CHROOCOCCUM
	per cent	*per cent*	*per cent*	*per cent*
Amide N...............................	13.42	20.36	18.25	17.90
Humin N...............................	13.18	15.82	13.67	9.62
Basic N:				
Arginine N............................	19.19	16.66	20.26	16.25
Histidine N...........................	0.16	0.16	0.17	0.11
Cystine N.............................	0.31	0.29	1.03	0.50
Lysine N..............................	7.24	5.11	4.51	3.14
Filtrate N:				
Amino N..............................	44.33	39.25	40.08	50.34
Non-amino N.........................	1.44	2.25	1.95	2.16
Recovery..............................	99.27	99.90	99.92	100.02
Tyrosine N............................	1.00	1.22	0.78	0.62
Tryptophane N........................	0.41	0.19	0.15	0.11

TABLE 4

The distribution of nitrogen in Azotobacter

(Results expressed as percentage of original material, air-dry basis)

	A. AGILIS	A. VINELANDII	A. BEIJERINCKII	A. CHROOCOCCUM
	per cent	*per cent*	*per cent*	*per cent*
Amide N...............................	1.340	1.832	0.720	0.716
Humin N...............................	1.312	1.425	0.540	0.385
Basic N...............................	(2.70)	(2.00)	(1.025)	(0.800)
Arginine N............................	0.95	0.75	0.40	0.325
Histidine N...........................	0.078	0.073	0.003	0.002
Cystine N.............................	0.034	0.026	0.037	0.020
Lysine N..............................	1.638	1.151	0.585	0.453
Filtrate N:				
Total N...............................	4.55	3.76	1.67	2.10
Amino N..............................	4.41	3.53	1.55	2.01
Non-amino N.........................	0.04	0.23	0.12	0.09
Recovery..............................	9.90	9.00	3.95	4.00
Total N (Kjeldahl method)..............	9.95	9.00	3.95	4.00

of lignin-like materials. The differences in the amounts of ether-soluble organic matter, alcohol-soluble organic matter, and water-soluble organic matter do not appear to be constant enough to warrant any generalizations.

The Van Slyke analysis did not reveal any constant variations. If, however, the results are expressed in terms of percentage of original material instead of percentage of nitrogen, as in table 4, the four species may be grouped, according to relationships, into the two groups mentioned in the foregoing. Because of the low nitrogen content, these differences are lost in table 3, for if the results are expressed in terms of total nitrogen, the variations are so magnified that the relationships are not apparent.

The results show, then, a decided similarity between *A. agilis* and *A. vinelandii* and between *A. beijerinckii* and *A. chroococcum*. This is in accord with the findings of Löhnis and Smith (17), who concluded from a study of cultural characteristics and life histories of many strains of Azotobacter that "only two species of Azotobacter are characterized thus far: *Azotobacter chroococcum* and *A. agile* Beij. (syn. *A. vinelandii* J. G. Lipman). *A. beijerinckii* Lipman is a variety of *A. chroococcum*, and *A. vitreum* Löhnis is probably a variety of *A. agile*. *A. smyrnii* C. B. Lipman et Burgess can not be accepted as a species; according to all marks ascribed to it by its authors, it is a large sporulating growth type of *A. chroococcum*. *A. hilgardii* C. B. Lipman and *A. woodstownii* J. G. Lipman are both incompletely described and should not be retained." Aso and Yoshida (1), by means of serum reactions (compliment fixation), have been able to distinguish various types of Azotobacter. Their results show that Azotobacter can be classified into three types: *A. chroococcum*, *A. vinelandii*, and *A. vitreus*. They found that *A. chroococcum* and *A. beijerinckii* are of the same type and that apparently the latter is a strain of the former. The author has shown elsewhere (10) that over a wide range of temperatures *A. agilis* and *A. vinelandii* were more active nitrogen fixers than *A. beijerinckii* or *A. chroococcum*. The ratio of nitrogen fixed is not proportional to the nitrogen content of the particular species, however, which would indicate that since *A. chroococcum* and *A. beijerinckii* have lower nitrogen content, they must have a higher growth rate. Many soil bacteriologists have considered *A. chroococcum* to be the most active species of the genus, but this is probably because few studies have been made in which all the species were compared, and also because *A. chroococcum* is more widely distributed than the other species

The results show, therefore, that *A. agilis* and *A. vinelandii* are closely related, and that a similar relationship exists between *A. chroococcum* and *A. beijerinckii*.

Larmour (16) has compiled the results of the analyses of many proteins. When the results of the Van Slyke determination are compared with those given by Larmour, the values for humin·nitrogen are much higher than any he cites, and the values for amide nitrogen are also quite high. It is probable that the carbohydrate fraction may be partially responsible for these results. Hydrolysis with hydrochloric acid may convert a portion of the carbohydrates to aldehydes, and a subsequent condensation with tryptophane would form humin (9). The high values for ammonia may be due either to deaminization of amino acids during hydrolysis or to the presence of free ammonia resulting

from the fixation of atmospheric nitrogen, and which had not been converted
into amino acids. It may be, however, that the nitrogen fixing organisms are
characterized by the presence of large amounts of amino nitrogen. In a study
of the proteins of the nodules of *Vicia faba*, Paresi and Masetti-Zannini (19)
report the following results: ammonia nitrogen, 18.80 per cent; humin nitrogen,
12.53 per cent; basic nitrogen, 16.91 per cent; non-basic nitrogen, 50.87 per
cent (recovery 99.11 per cent).

Arginine and lysine are the principal amino acids of the basic fraction. Small
amounts of cystine and histidine are also found. The non-basic fraction con-
tains the larger part of the amino acids, and, unfortunately, satisfactory methods
are not available for determining them. These amino acids are probably
glycine, alanine, valine, phenylalinin, and other amino acids.

The tyrosine content of these organisms is very small, and it is interesting
to note that *A. chroococcum* contains the smallest amount of the four species.
Rippel and Ludwig (21) have claimed that the black pigment formed by *A.
chroococcum* is due to the conversion of tyrosine and melanin. The stock
culture of *A. chroococcum* was selected because of the intense pigment that it
formed. Ranganathan and Norris (20) were not able, however, to demonstrate
the presence of tyrosinase in a culture of *A. chroococcum*. If pigmentation of
this species is due to the conversion of tyrosine to melanin, it is difficult to
explain why the other species do not form pigment so readily under identical
conditions, since they all contain at least as much tyrosine as does *A. chroococ-
cum*, unless the enzyme tryrosinase is found only in *A. chroococcum*. This
is particularly true in the case of *A. beijerinckii*, which appears to be very
closely related morphologically, culturally, serologically, and chemically to
A. chroococcum.

The results given in the Van Slyke analysis do not agree well with those
given by Omeliansky and Sieber (18) for *A. chroococcum*. These investigators
used a different agar and a longer incubation period. These factors, together
with a different method of obtaining material and subsequent modifications of
analytical methods, may account for the differences. The author realized at
the beginning of this investigation that there are many objections and limita-
tions to the Van Slyke method, but it appeared to be the best method available.

In general, the beijerinckii-chroococcum group contained larger amounts of
water soluble organic matter than the agilis-vinelandii group. This consisted
chiefly of protein and materials which gave a positive test with Molisch's rea-
gent but which did not reduce Fehling's solution or give a positive test for
starch. This carbohydrate fraction may be the gum which Hamilton (13)
has described for *A. chroococcum*. Further tests were not made upon this
fraction.

Within recent years, studies upon respiration have shown that substances
which may act as hydrogen acceptors or donors exist within animal and bac-
terial cells. In the animal body, glutathione plays an important part in the
oxidation-reduction system. Callow and Robinson (6) have reported the

presence of glutathione in many species of bacteria, and although their results have been questioned, there exists in bacteria some substance capable of giving a positive test with sodium nitroprusside. This test has been considered specific for the sulfydryl ($-SH$) group, but it is now recognized that other substances may give a positive test with this reagent.

In order to determine whether Azotobacter contained any substance capable of acting as a hydrogen acceptor or donor, the material which had been used in the other determinations was tested for glutathione, by the method of Callow and Robinson (6). Each species of Azotobacter gave a positive reaction. Although this test alone is not sufficient evidence to verify the presence of glutathione in Azotobacter, the conclusion does not seem improbable, and possibly a part of the cystine found in the Van Slyke analysis might result from the oxidation of cysteine, which is a part of the glutathione molecule.

The mechanism of protein synthesis by Azotobacter

Space will not permit an extensive discussion of the mechanism of protein synthesis, especially since such a discussion must be hypothetical. It is now generally recognized that the first product of nitrogen fixation by Azotobacter is ammonia. The ammonia is then converted into amino acids by combining with various organic acids: with acetic acid, glycine is produced; with propionic acid, alanine is produced; with phenyl-hydroxy-propionic acid, tyrosine results; and in a similar way other amino acids might be formed.

The nitrogen fixed by these organisms comes from the atmosphere; hydrogen cannot, since it occurs in the air in extremely small amounts. Hydrogen is therefore obtained during the breakdown of carbohydrates. In this case, Kluyver's (15) theories of respiration and photosynthesis, involving hydrogenations and dehydrogenations, are applicable and may be represented by the following equation:

$$X \text{ (Catalysts)} + H_2A = XH_2 + A$$
$$(A \text{ may be O, S, or nothing})$$
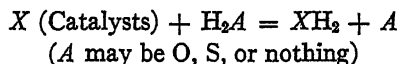
In the case of nitrogen fixation, the equation would be:

$$X + N_2 = 2XN$$
$$XN + H_2A = XNH_2 + A$$
$$XNH_2 + 2H = NH_3 + X$$
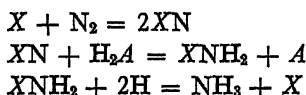
This is essentially the same mechanism as that suggested by Blom (4). He assumes that organic iron compounds are the catalysts.

Recent studies in carbohydrate metabolism, both animal and microbiological, have suggested a mechanism of carbohydrate breakdown which is very similar. Space will not permit a discussion of these theories, which are fully discussed by Buchanan and Fulmer (5), Hardin (14), Kluyver (15), and Stephenson (22). In general, the sugar molecule (hexose) is converted into methyl-glyoxal (or some activated form of it) and into pyruvic acid. From this stage, it may

be converted by hydrogenations and dehydrogenations into various acids; the carbon chain may be shortened by decarboxylation or lengthened by condensations.

This postulated mechanism of protein synthesis may be summarized as follows: In the process of carbohydrate breakdown, various organic acids are formed, and the active hydrogen produced in the processes is utilized by the organisms for the fixation of nitrogen (probably a direct union to form NH_3). Ammonia reacts with the respective organic acids to form amino acids, which are subsequently converted into protein.

Table 2 shows that Azotobacter are able to synthesize rather complex carbohydrate materials. There may be a relationship between protein synthesis and gum production by these organisms. Hamilton (13) has shown that the amount of gum produced by *A. chroococcum* was not greatly affected by simple carbohydrates, but that polysaccharides led to increased gum production. Gum is not formed in peptone solutions. Since this gum does not contain nitrogen, its production may be associated with protein synthesis, probably in the production of active hydrogen for fixation. The decomposition of carbohydrates liberates energy for these organisms. If, however, small amounts of nitrogen are fixed, as a result of the utilization of nitrogen compounds, small quantities of activated hydrogen are required, which means low respiration and small amounts of available energy. Gum formation by Azotobacter may be considered as a sort of "luxury synthesis." This may account for the difference in hemicellulose content of these organisms. *A. agilis* and *A. vinelandii* fixed larger amounts of nitrogen, hence more energy was available and larger amounts of hemicellulose were synthesized. The energy produced from carbohydrates in excess of growth requirements is probably used in the production of complex carbohydrates.

SUMMARY

Four species of Azotobacter were grown on nitrogen-free mannitol agar, and the bacterial growth was analyzed.

A. vinelandii and *A. agilis* are very similar in composition; a close similarity exists between *A. chroococcum* and *A. beijerinckii*. This relationship also exists in their nitrogen fixing abilities. A Van Slyke distribution did not reveal consistently wide differences but did indicate this similarity.

Arginine and lysine were the amino acids found in largest amounts. Tyrosine, tryptophane, cystine, and histidine were found in smaller amounts. Approximately 40 per cent of the total nitrogen was found in the non-basic fraction, which indicates the presence of simpler amino acids (glycine, alanine, etc.).

Qualitative tests showed the presence of a substance giving a positive reaction with sodium nitroprusside. This substance may be glutathione.

Semi-quantitative determinations indicated that the proteins present were chiefly globulins, glutelins, and albumins.

The metabolism of Azotobacter and the formation of amino acids are discussed briefly.

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FACTORS INFLUENCING PHOSPHATE FIXATION IN SOILS

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Though phosphate fixation in soils has been studied ever since Way, in 1850, discovered its existence, yet our knowledge of it is still fragmentary. The present paper is a report of the study of some of the factors concerned in fixation of phosphate, with some discussion. Knowledge of fixation is necessary to intelligent use of fertilizers with respect to amount, kind, and most effective placement of the phosphate.

In this paper, the term "fixation" designates the removal of PO_4 from a water solution by the soil. Numerous other materials also have the power of fixation. When PO_4[1] has been fixed by soil or other material, it is no longer easily soluble in water and may not be easily soluble in such relatively weak solvents as are commonly used to measure the availability of soil phosphate.

Ford (4, p. 6) says "Fixation is the term applied to the phenomenon whereby soluble phosphorus is removed from solution by soils. It is often designated by other terms such as 'absorption,' 'adsorption,' or 'reversion'."

The work here reported was done for the purpose of obtaining more detailed and exact information on the subject of fixation by means of chemical laboratory experiments. It was sought to learn how fixation is influenced by a variety of treatments mostly confined to the effects of water alone or with small amounts of added salts, such as may occur in the soil solution. Fixation caused by iron or aluminum compounds as such was very little studied. This has been fairly well cleared up by the studies of Gile (9), Weiser (22), Ford (4), Heck (10), Gaarder (7), Dean (1), and others.

Some of the experiments were not carried far enough or were not repeated sufficiently to furnish a basis for entirely valid conclusions. But the results obtained are suggestive of further experimental work which might reveal facts of much importance. The writer may not continue the work, therefore, he offers this report as suggestive rather than conclusive.

No complete search of the literature has been made in connection with this study. Some of the recent papers have been examined, but most of the experimental work was done before some of the important recent papers were in print.

The papers of Gile (9), Demolon (2), Thomas (21), McGeorge and Breazeale

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[1] In this paper the expression "PO_4" is meant to include any one or all of the phosphate ions, PO_4^{---}, HPO_4^{--}, or $H_2PO_4^{-}$.

(15), Dean (1), Ford (4), Fraps (5), Fudge (6), Gaarder (7), and Heck (10) have been reviewed.

To summarize the work and opinions of these ten workers, it may be said that all of them believe that pH and degree of saturation of the soil with PO_4 are of greatest importance in all soils. Most of them consider that hydrated oxides of Fe and Al are the chief causes of fixation in non-alkaline soils. Four find that easily soluble silicates increase the availability of PO_4. Although easily soluble Ca and Mg in soil quickly fix soluble phosphates, the insoluble compounds formed are fairly available to plants in non-alkaline soils. But McGeorge and Breazeale find that in calcareous soils, basic very insoluble and non-available phosphates are formed. Demolon considers that anion exchange is the cause of much of the increased solubility of PO_4 produced by addition of soluble silicates, and this view is in accord with the point made by Clarens, and quoted by Thomas, that the clay molecule contains a basic OH group replaceable by acid radicals, such as PO_4. Most of them agree that organic matter, especially soluble organic matter, increases the solubility and penetrating power of soil phosphate. Also, organic compounds of P are much less fixed than inorganic. McGeorge and Breazeale, the only ones who worked much with calcareous soils, think that the unavailability of PO_4 in these soils is due not only to high pH in which phosphates of Ca and Mg are insoluble but still more to formation of very insoluble basic carbonate-apatite.[2]

EXPERIMENTAL

The general character of the soils used in the experiments, with respect to phosphate supplying power, is presented in table 1.

The subject studied was under the topics:

1. Measurement
2. Phenomena
3. Causes ⎬ of fixation
4. Control

Measurement

Some means of measuring the fixing power of a soil seems necessary to an intelligent study of the subject.

A review of the procedures suggested by Heck (22, p. 20), Lohse (12), McCool (14), Gile (9), Ford (4), Fraps (5), and Fudge (6) reveals that all of them are empirical, and that no one method has been generally or officially accepted. What is wanted is a method that will give reliable and reproducible results with least expense of time, labor, equipment, and money. The one adopted herein, chiefly on account of convenience and rapidity, consists in adding a soluble phosphate to a mixture of soil and water, agitating for one hours, filtering, and in the filtrate determining the phosphate by the molyb-

[2] A more extended review of the papers of these investigators has been omitted in order to save space.

denum-blue method. Two variations have been used. The same amount of phosphate is added to all soils, in which case the PO_4 in the extract may be extremely variable, or a variable amount is added, according to the fixing power of the soil, so that the extract will always have about the same concentration of PO_4, e.g. 1 p.p.m.

Soils differ greatly in fixing power; consequently, in using the first method, unless a relatively large amount of PO_4 is added to all soils, the fixing power of high fixing soils will not be represented in proper proportion to that of low fixing soils. This is shown in table 2 with soils 59 and 35, both of which fix 99+ per cent of added PO_4, though the highest addition to soil 59 contains sixteen times as much PO_4 as the lowest. On the other hand, if enough PO_4 is added so that the extract will contain 1 p.p.m., or any other arbitrary concentration, the amounts thus required will much better indicate the relative

TABLE 1

General character of soils used in fixation experiments

			PO₄		
SOIL NO. AND NAME	FIXING POWER	pH	Truog	Water extract	
			p.p.m. in soil	1:1 p.p.m. in solution	1:100 p.p.m. in solution
1c. Yolo silty clay loam..........	40	6.7	620	0.20	0.18
30. Fresno fine sandy loam.........	0	6.9	440	5.00	0.40
35. Holland loam.................	170	5.4	20	0.04	0.03
37. Nord sandy loam..............	...	8.2	400	0.58	0.18
38. Vina clay loam................	80	7.2	248	0.18	0.21
53. Delhi sand....................	0	7.1	88	1.32	0.27
59. Aiken clay....................	460	6.0	4	0	0
64. Vina silt loam................	70	6.9	160	0.10	0.08
78. Gold Ridge sandy loam........	35	4.8	20	0.20	0.06
95. Ramona loam.................	7	8.3	144	0.80	0.24

fixing power of different soils. In other words, *if the amount necessary to give the desired concentration in the extract is taken as a measure of fixing power*, instead of the amount actually fixed, a more reliable estimate of the character of the soil is obtained. In figure 1, the graphs plotted from table 2 for soils 59 and 35 show that an increasing proportion of the PO_4 added remains in solution as the amount added increases, indicating that no definite compound is formed. Gile (9) observed the same thing.

In practice, the second method usually requires more than one trial to find the amount needed, so that three 100-gm. portions of air-dry soil are mixed with three 100-cc. portions of water containing three quite different amounts of PO_4. The mixtures, placed in 300-cc. bottles, are shaken occasionally for one hour, then filtered, and the PO_4 in the filtrate is determined. From the three figures found may be calculated the amount that must be added in order

to obtain an extract containing 1 p.p.m. PO$_4$. This amount in milligrams per kilogram of soil, is called the "fixing power" of the soil. Some soils may contain that or a greater amount without any added PO$_4$. These soils, as well as those to which it has been added, may still be able to fix large quantities of PO$_4$.

TABLE 2

Fixation of PO$_4$ by soils from different amounts added to 100 gm. soil

SOIL NO.	PO$_4$ ADDED	PO$_4$ REMAINING IN SOLUTION	PER CENT FIXED OF AMOUNT ADDED	FIXING POWER—PO$_4$ PER KGM. OF SOIL
	mgm.	*p.p.m.*		*mgm.*
59	5	0.06	99.9	
	10	0.12	99.9	
	20	0.18	99.9	
	40	0.80	99.9	460
	50	1.16	99.8	
	60	3.20	99.5	
	80	3.34	99.6	
64	5	0.66	98.7	
	7	1.00	98.6	70
	10	1.44	98.6	
38	5	0.48	99.0	
	8	1.00	98.7	80
	10	1.14	98.8	
78	3	0.72	97.6	35
	4	1.24	96.9	
	5	1.34	97.3	
	10	4.00	96.0	
35	5	0.16	99.7	
	10	0.32	99.7	
	15	0.64	99.5	170
	20	1.76	99.1	
	40	8.00	98.0	
1c	3	0.88	97.1	
	4	1.00	97.5	40
	5	1.60	96.8	
	10	2.50	97.5	

In order to make the determination of fixing power more similar to the effect of adding phosphate to soil in the field, 100 gm. of soil was placed in a 48 mm. diameter percolator tube and, after addition of PO$_4$, was leached with water or other solvent. Slight differences in preparation of the setup make such great differences in the rate of percolation and in the concentration of PO$_4$

in the percolate, that the results are, in most cases, not reproducible. When the phosphate was applied in a solution of 100 p.p.m. concentration, the PO_4 appeared in the percolate after most of the fixing power of the soil had been satisfied. This may require several times as much PO_4 as in an equilibrium
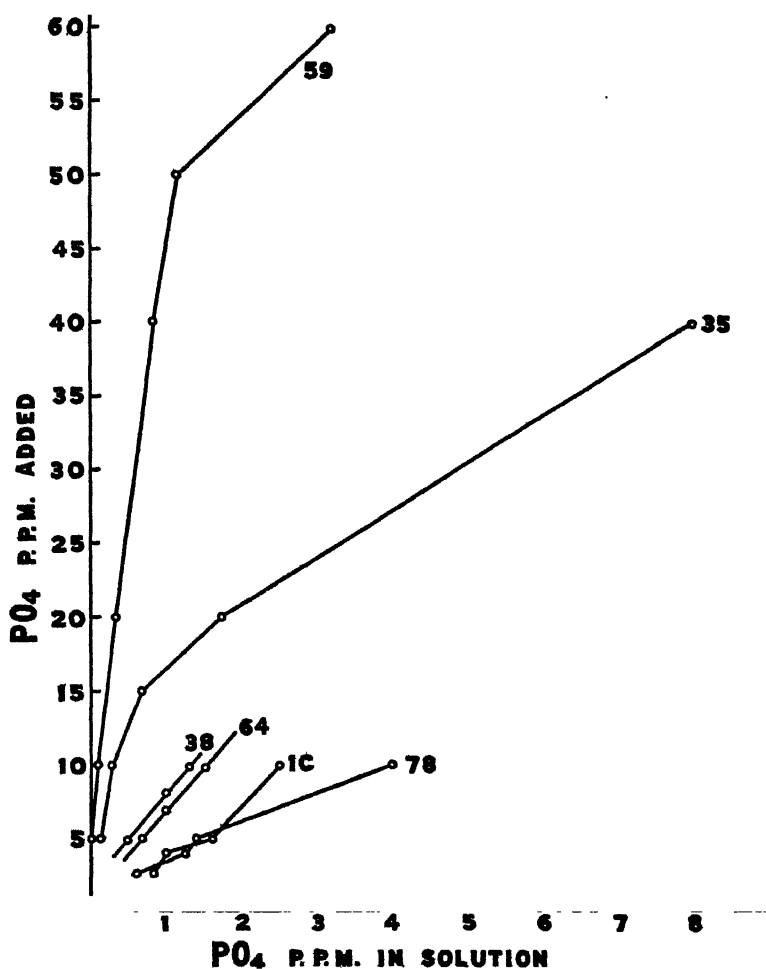
FIG. 1. FIXATION OF PO_4 FROM DIFFERENT AMOUNTS ADDED TO 100 GM. SOIL

extract and may be a very tedious procedure. The first 100 or 200 cc. of percolate may contain practically no PO_4. In table 3 are recorded the results obtained on a few soils by this method. It is so difficult to control and to produce duplicate results that this percolation method is considered very unsatisfactory for measuring fixing power. When the phosphate was applied as a dry

salt on top of the column of soil in the percolator, the results are still less useful. The leaching water or the dilute solution applied soon removes nearly all the soluble matter from the soil so that percolation nearly or entirely ceases. If some electrolyte is added with the water, percolation may be kept up, but the solubility of the PO_4 is so changed that results are of little value.

A serious difficulty with all methods for determining fixing power of a soil is that *soils have no definite fixing power*. Figure 1 and table 2 reveal that the larger the amount of PO_4 added, the less firmly is it held by the soil against the solvent action of water. Gile (9) reports similar results. Later in this paper, it will be shown that much of the added PO_4 which has apparently been rendered insoluble by the soil may be washed out by much water or by a dilute solution of Ca. Since the solubility of the PO_4 in the soil-PO_4 complex is very low, much water is needed to wash out the fixed PO_4 from the soil. Except

TABLE 3

PO_4 in successive percolates from different amounts added to 100 gm. soil

SOIL NO.	PO₄ ADDED	PO₄ IN PERCOLATE NUMBERS							TOTAL
		1	2	3	4	5	6	7	
	mgm.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1c	0	0.06	1.50	2.00	1.44	1.32	0.64	0.48	0.74
	5	0.12	1.66	2.28	2.00	2.00	1.76	1.76	1.16
	10	0.24	6.60	5.40	4.40	2.84	1.76	1.60	2.28
	20	8.00	30.80	13.50	9.00	6.16	3.20	2.68	7.33
64	0	0.24	0.72	0.88	0.88	0.84	0.64	0.56	0.48
	10	0.08	0.88	0.88	0.64	0.96	0.56	0.56	0.47
	25	3.64	7.20	5.40	5.00	3.64	2.28	2.68	2.98
	50	10.00	33.50	15.50	12.00	6.16	4.00	3.64	8.28

when the dilution is great, the soil has greater power to retain the PO_4 than the water has to dissolve it away from the complex.

Fixation of PO_4 by a soil is a time reaction which may continue for months, the rate being influenced by the proportion of water to soil. Scarseth and Tidmore (18, p. 141) found that the reaction between PO_4 and colloidal clay was practically instantaneous and (19, p. 154) that the PO_4 added to two clay soils in pots was nearly all fixed within one year.

Table 4 and figure 2 present figures showing the rate of fixation in soils kept at optimum moisture for one year, and table 5 shows the increase in fixation in a 1:1 water suspension during 20 days. The gradual decrease of water-soluble PO_4 may be partly because no definite compound, but only a loose complex, is formed, and partly because the added PO_4 makes imperfect contact with the soil particles. Perhaps 90 per cent of the PO_4 added to a soil-water mixture may be fixed in one hour (see table 5). Thus the rate of fixation is decreased in two ways, by diminishing the avidity of the soil for PO_4 and also

TABLE 4

Time effects in fixation at optimum moisture

Treatments: A = $CaH_4(PO_4)_2$, B = $CaHPO_4$, C = $(NH_4)_2HPO_4$

SOIL NO.	TIME	PO₄ IN EXTRACTS								
		Truog Extracts			1/100 Water			1/1 Water		
		A	B	C	A	B	C	A	B	C
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1c	1 hour	2.00	2.00	2.00	0.72	0.62	0.58	2.66	0.28	1.14
	1 day	2.20	2.20	1.82	0.58	0.54	0.44	1.26	0.62	0.66
	7 days	2.00	2.00	2.00	0.34	0.32	0.28	0.72	0.50	0.48
	21 days	2.00	2.00	2.00	0.36	0.26	0.28	0.80	0.72	0.62
	2 months	1.60	1.60	1.60	0.20	0.18	0.20	0.88	0.62	0.62
	6 months	2.00	1.82	1.66	0.24	0.22	0.20	0.88	0.44	0.40
	12 months	1.14	2.00	2.00	0.16	0.20	0.20	0.58	0.48	0.50
35	1 hour	0.88	0.80	0.58	0.88	0.80	0.50	1.60	1.14	0.58
	1 day	0.80	0.62	0.44	0.40	0.32	0.20	0.44	0.50	0.20
	7 days	0.58	0.48	0.40	0.14	0.12	0.08	0.16	0.16	0.12
	21 days	0.50	0.40	0.30	0.08	0.08	0.08	0.20	0.12	0.04
	2 months	0.32	0.24	0.18	0.10	0.08	0.08	0.08	0.08	0.04
	6 months	0.40	0.26	0.20	0.04	0.08	0.06	0.12	0.08	0.06
	12 months	0.28	0.30	0.20	0.08	0.10	0.04	0.04	0.08	0.08
37	1 hour	2.00	1.60	1.60	1.34	0.20	1.00	6.66	0.54	8.00
	1 day	1.60	1.42	1.66	0.88	0.24	0.62	6.66	0.72	6.66
	7 days	1.82	1.60	1.42	0.66	0.24	0.58	6.66	1.60	5.00
	21 days	2.00	1.60	1.60	0.66	0.24	0.50	6.66	2.22	4.44
	2 months	1.76	1.34	1.60	0.50	0.24	0.32	5.80	2.50	3.34
	6 months	1.82	1.42	1.42	0.58	0.30	0.28	6.66	2.00	2.26
	12 months	1.48	1.60	1.48	0.40	0.28	0.36	4.00	2.66	2.50
59	1 hour	2.00	1.60	1.00	2.22	1.60	1.00	2.86	2.36	0.88
	1 day	1.76	1.34	0.66	1.00	1.00	0.48	1.34	1.42	0.50
	7 days	1.14	0.72	0.54	0.48	0.40	0.16	0.62	0.54	0.18
	21 days	1.00	0.62	0.34	0.40	0.24	0.12	0.28	0.24	0.08
	2 months	0.58	0.50	0.20	0.12	0.14	0.04	0.12	0.10	0.06
	6 months	0.54	0.26	0.14	0.08	0.04	0.04	0.08	0.10	0.04
	12 months	0.30	0.28	0.16	0.08	0.08	0.04	0.12	0.04	0.08
64	1 hour	1.34	1.50	1.50	1.00	0.72	0.72	4.00	1.76	1.82
	1 day	1.50	1.14	1.26	0.66	0.50	0.50	2.00	1.34	1.14
	7 days	1.42	1.14	1.34	0.48	0.32	0.32	1.60	0.62	0.54
	21 days	1.34	1.00	1.14	0.36	0.20	0.24	1.14	0.48	0.34
	2 months	0.88	0.88	1.00	0.20	0.18	0.16	0.54	0.26	0.20
	6 months	1.34	1.00	1.00	0.20	0.14	0.14	0.44	0.20	0.16
	12 months	1.00	1.00	1.06	0.20	0.20	0.16	0.28	0.22	0.20
78	1 hour	0.50	0.28	0.44	0.50	0.32	0.32	2.22	1.00	0.80
	1 day	0.32	0.20	0.20	0.26	0.12	0.14	0.80	0.50	0.36
	7 days	0.30	0.20	0.20	0.16	0.08	0.12	0.44	0.14	0.18
	21 days	0.20	0.12	0.16	0.08	0.08	0.08	0.40	0.16	0.14
	2 months	0.20	0.16	0.14	0.08	0.08	0.08	0.28	0.16	0.10
	6 months	0.22	0.16	0.16	0.08	0.08	0.08	0.24	0.10	0.10
	12 months	0.10	0.20	0.10	0.08	0.08	0.08	0.18	0.12	0.12

by lowering the concentration of the uncombined PO_4 remaining in the solution. Perhaps, if the concentration in the solution were kept constant, which is very difficult to do, the rate of fixation would be constant for some time. After 90 per cent of the PO_4 is fixed, five or ten hours may be needed to fix 90 per cent of the remainder, thus leaving only 1 per cent of the original still in solution, so
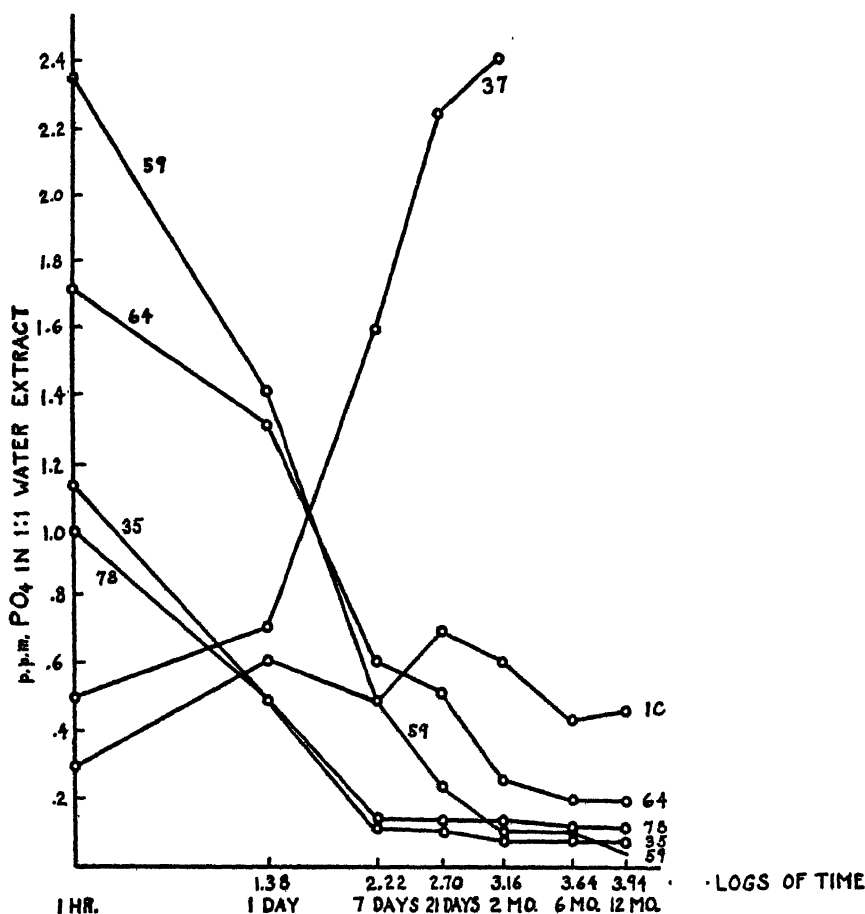
FIG. 2. CHANGE, DURING ONE YEAR, IN PO_4 IN 1:1 WATER EXTRACT OF SOILS TO WHICH WAS ADDED $CaHPO_4$

that after that the rate of fixation becomes very slow. A consequence of this time factor in fixation is that in order to obtain comparable results in any short laboratory method for measuring fixation, it is necessary to hold to a constant time during which fixation is allowed to take place. Apparently two soils having the same total fixing power may have quite different rates of fixation, on account of physical and chemical differences. The results given in table 5

show that in a 1:1 soil-water mixture, most of the fixation takes place within one hour. Since the procedure is empirical, one hour has been selected as long enough for nearly complete fixation and short enough for a convenient laboratory test.

To learn something of the rate of fixation and the length of time during which fixation takes place, the following experiment was made.

Fixation of PO₄ during one year in soil at optimum moisture.—The air-dry soils were mixed with the dry powdered phosphates, then moistened to optimum by atomizing the water onto them, and stored in covered glass jars. After

TABLE 5

Time factor in fixation from 1 minute to 20 days, PO₄ in 1:1 equilibrium extracts

TIME	FILTRATE NO.	PO₄ IN FILTRATES			
		Soils...........1c PO₄ added, mgm. 4	38 8	64 8	78 4
		mgm.	*mgm.*	*mgm.*	*mgm.*
1 hour	1	0.33	0.44	0.29	0.31
	2	0.88	0.80	0.88	0.50
	3	1.25	1.18+	1.25	0.63
5 hours	1	0.22	0.24	0.12	0.11
	2	0.50	0.57	0.40	0.20
	3	1.00	1.00	0.62	0.33
24 hours	1	0.22	0.36	0.13	0.06
	2	0.57	0.63	0.33	0.12
	3	1.11	1.00	0.54	0.25
5 days	1	0.25	0.16	0.06	0.04
	2	0.29	0.25	0.08	0.07
	3	0.44	0.36	0.14	0.10
20 days	1	0.12	0.16	0.06	0.04
	2	0.13	0.18	0.06	0.04
	3	0.29	0.25	0.08	0.04

some months, more water was added in order to bring the soils to the original water content. When the soils were to be sampled for analysis, the contents of the jars were well mixed before the desired portion was removed. This procedure somewhat resembles the conditions in field soils, but is not so favorable to rapid fixation as were most of the other experiments described in this paper where the soils were mixed with much more water.

Three different phosphates were used in quantity to supply the same amount of PO₄ to each jar of the same soil, but different for the different soils, with the intention that the amount added would produce a concentration of 1 p.p.m. in the 1:1 water extract at the start. The results recorded in table 4 show

that there was much departure from this. The same amount of PO_4 from different salts did not produce the same level of PO_4 in the extract.

In the following the progress of fixation, in order to obtain a better idea of the availability of the PO_4 three different extracts were made from each soil each time they were analyzed. The figures given in table 4 reveal some irregularities, perhaps partly due to inaccuracies in estimation of the PO_4 in the solutions. However, the general decrease in solubility with time is apparent, with the exception that there was an increase in the PO_4 in the 1:1 water extract of soil 37 + $CaHPO_4$. No explanation of this variation is apparent. It is evident that the amount of PO_4 fixed in a unit of time was much greater at first and gradually decreased during the year of the experiment. In figure 2 are plotted the results from the 1:1 water extract of soils + $CaHPO_4$ (given in table 4), in which the logarithms of the time units are used in order to compress the graphs into the limits of a single page. Probably a large part of the decrease in rate of fixation was caused by the great decrease of water-soluble PO_4 remaining in the soil as fixation progressed.

The amount of PO_4 fixed from the different salts was different, as indicated by the water extracts, though the acid Truog extract shows less difference. The acid $CaH_4(PO_4)_2$ was least fixed, and the neutral $CaHPO_4$ most fixed in the majority of cases, yet in some cases the very soluble ammonium phosphate was most fixed. It appears, therefore, that the relative fixing power of different soils is different for different salts, thus indicating that the most effective salt for one soil may not be the best for a different soil. The colorimetric method used in estimating PO_4 in the extracts is not capable of exact estimates of the concentration when it is below 0.1 p.p.m. in the solution, so that some variation in the smaller amounts found is not significant.

The experiment demonstrates that most of the added PO_4 was fixed within a few hours and that fixation may continue for a year or more.

Another simple experiment gives some idea of the speed of fixation. To each of four bottles containing 100 cc. water with 5 mgm. PO_4 in each were added 50 gm. soil. After being shaken for various lengths of time, the mixtures were filtered and PO_4 was determined in the extracts, with the following results:

Time before filtering .... *1 min.*	*10 min.*	*1 hour*	*8 hours*
PO_4 in extract Soil 38, p.p.m. 2.00	1.44	1.16	0.88
PO_4 in extract Soil 64, p.p.m. 4.44	2.40	1.32	0.88

The greater the volume of water mixed with a soil, the greater will be the amount of PO_4 remaining in solution (table 6). In many cases, the soil extract will have almost the same concentration of PO_4 whether the ratio soil-water is 1/1 or 1/100. In other cases, when there is much easily water-soluble PO_4, the concentration of the PO_4 diminishes rapidly as the dilution increases (soils 30 and 53, table 6). In this case, since the phosphate is easily soluble, the solution is not saturated except at small dilutions. In the former case, the phosphate complex is so slightly soluble that the solution is saturated with it

TABLE 6

Increase in dissolved PO₄ with increased dilution in equilibrium water extract

SOIL NO.	DILUTION RATIO SOIL:WATER	PO₄ ADDED TO SOIL	PO₄ IN EXTRACT
		p.p.m.	*p.p.m.*
64	1/2	100	1.82
	1/5		2.10
38	1/2	100	1.25
	1/5		1.60
30	1/1	0	5.70
	1/5		3.60
35	1/2	200	1.00
	1/5		1.14
53	1/1	0	4.44
	1/5		1.34
59	1/2	400	0.62
	1/5		0.58
78	1/1	40	0.88
	1/5		0.88

TABLE 7

Fixation of PO₄ by various sizes of soil particles

SOIL	PARTICLE SIZE	PER CENT OF WHOLE	TRUOG AVAILABLE	FOR 20 GM. MATERIAL	
				PO₄ Added	PO₄ Fixed
				m̄ḡm.	*m̄ḡm.*
35	On 1-mm. sieve	12.2	20	2.5	1.62
	On 40 mesh	17.4	28	2.5	2.10
	On 100 mesh	18.2	36	2.5	2.42
	Through 100 mesh	48.5	56	2.5	2.48
77	On 1-mm. sieve	12.0	64	1.4	1.13
	On 40 mesh	17.3	80	1.4	1.17
	On 100 mesh	31.7	112	1.4	1.29
	Through 100 mesh	39.0	232	1.4	1.34
78	On 1-mm. sieve	0.4	...	...	.....
	On 40 mesh	0.7	...	...	.....
	On 100 mesh	13.2	48	0.8	0
	Through 100 mesh	86.7	48	0.8	0.24
95	On 1-mm. sieve	11.2	32	0.2	0
	On 40 mesh	11.3	48	0.2	0.024
	On 100 mesh	19.5	80	0.2	0.024
	Through 100 mesh	47.8	248	0.2	0.068

except at very high dilution. A consequence of these facts is that in making a test of fixing power, the ratio of soil to water must be held constant in order to obtain comparable results with different soils.

The physical character of the soil greatly influences fixation but, since this must be accepted as it is found, it need not be considered in measurement of fixation, except to see that the sample fairly represents the soil in question. If the soil is sandy and coarse, it may easily segregate into separates of different degrees of fineness which will have quite different fixing power. Figures in table 7 illustrate this very well, showing the great variations in fixing power of coarse and fine particles of four soils.

Phenomena of fixation

Time of contact, ratio soil:water, and physical character have much influence; therefore, in measuring fixation these factors must be considered and kept as nearly constant as possible. Since they have been discussed at some length under the preceding section, they may be omitted here with the reminder that they are important.

In most of the work here reported with equilibrium extracts, they were made by mixing the soil, water, and other materials in bottles, which were shaken vigorously every few minutes for an hour, then filtered on büchner filters with suction. The first portion of the filtrate, usually not clear, was returned to the filter so that a clear filtrate was obtained. The PO_4 concentration was measured by the molybdenum blue method, according to Parker and Fudge (17). In most cases where successive percolates or filtrates were made, they were obtained by adding 100 cc. of the solvent at a time to the soil on the büchner after the water of the first mixture had been sucked out as much as possible. In each case, the filter was transferred to a clean bottle to collect the new filtrate.

In most cases, the added phosphate was a water solution of KH_2PO_4 of which 1 cc. = 1 mgm. or (sometimes 10 mgm.) PO_4. The results reported and the conclusions reached in this paper are mostly based on work with equilibrium extracts. Numerous attempts to obtain useful results by means of percolation extracts produced contradictory figures, as already referred to. Perhaps, in order to obtain reliable information, it may be necessary to carry the work into the field and operate with soils in place.

Solubility in water and in salt solutions.—Though there is great difference in the solubility of the PO_4 in different soils, it is generally true that up to three or four successive filtrates of 100 cc. each from a single portion of soil, the concentration of PO_4 increases, while, at the same time, the concentration of Ca diminishes. This is shown in many of the tables, and seems to indicate that removal of easily soluble Ca releases more PO_4 to enter solution, or that concentration of PO_4 in the solution of those soils is largely governed by the concentration of Ca. The pH of the successive extracts remains nearly constant (not shown in the tables). A decrease in pH is generally accompanied by an increase of PO_4 in the solution.

Removal of soluble matter by washing the soil usually causes deflocculation to such an extent that filtration becomes very slow. In order to escape this difficulty, a solution containing about 10 p.p.m. Ca + 5 p.p.m. Mg as sulfates has been much used in this work. With this wash, it is possible to continue percolation almost indefinitely. This concentration of Ca and Mg, however, is enough to repress the solubility of PO_4 considerably.

The effect on solubility of PO_4 of the common cations of soil solutions was studied with these elements present in equivalent amounts, about 2 m.e. in

TABLE 8

Effect of 2 m.e. of several cations, etc., on PO_4 in 1:1 equilibrium extracts of soils

K_2HPO_4, in amounts indicated, was added to all except those in column 4, water only

			PO₄ IN EXTRACTS										
1 SOIL	2 PO₄ ADDED	3 FILTRATE, NO.	4 Water only No PO₄	5 Water + PO₄	6 Ca 11 mgm.	7 Mg. 6.8 mgm.	8 K 21.8 mgm.	9 Na 12.8 mgm.	10 NH₄ 10.1 mgm.	11 CaCO₃ 0.5 gm.	12 0.5 gm. CaCO₃ + 10.1 mgm. NH₄	13 MgCO₃ 0.5 gm.
	mgm.		p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
78	4	1	0.06	0.64	0.20	0.44	0.48	0.64	0.80	0.06	0.20	0.12
		2	0.10	1.16	0.29	0.52	0.64	0.88	1.00	0.08	0.24	0.14
		3	0.14	2.00	0.40	0.72	1.44	3.20	2.16	0.06	0.24	0.16
		4	0.14	2.00	0.57	0.88	1.76	2.84	2.28	0.06	0.24	0.16
1c	4	1	0.20	1.16	0.50	1.00	0.80	1.16	1.00	0.20	0.62	0.50
		2	0.29	2.68	0.57	1.60	0.64	3.52	1.24	0.29	1.14	0.58
		3	0.57	4.00	0.80	3.20	3.20	5.32	3.68	0.29	1.60	0.66
		4	0.67	5.00	1.33	4.72	4.72	5.32	4.72	0.29	1.82	0.28
38	8	1	0.12	0.64	0.80	0.64	0.88	1.00	1.16	0.22	0.80	0.34
		2	0.50	1.32	1.16	1.76	1.60	2.00	1.32	0.29	0.88	0.38
		3	0.88	1.24	2.00	2.84	2.00	4.00	2.28	0.29	1.00	0.40
		4	1.18	1.16	2.28	2.28	2.28	2.68?	2.96	0.29	1.00	0.48
64	8	1	0.06	0.56	0.40	0.48	0.48	0.24	0.48	0.08	0.62	0.18
		2	0.12	2.16	0.80	0.88	0.72	1.60	1.44	0.08	0.66	0.16
		3	0.20	2.84	1.60	1.32	1.60	2.68	2.28	0.08	0.72	0.18
		4	0.25	3.82	1.44	1.60	2.28	2.68	2.28	0.08	0.72	0.18

100 cc., as sulfates. Results are given in table 8. It appears that solubility of PO_4 increases in the order Ca, Mg, K, Na, NH_4. With Ca ion, solubility of PO_4 is one-quarter of that in pure water, whereas with Na and NH_4, it is about the same as with water only.

The effect of $CaCO_3$ and $MgCO_3$ is also shown in table 8. $CaCO_3$ is more depressing than $MgCO_3$, but both greatly reduce solubility of PO_4. When NH_4 is added, PO_4 becomes more soluble even in the presence of $CaCO_3$ or $MgCO_3$. The NH_4 ion generally increases the solubility of PO_4 as shown in table 8 and

other tables. When Na and K are added with NH₄, the solubility of PO₄ is still more increased.

Soluble silica, added as Na_2SiO_3, seems always to increase solubility of PO₄, as shown in table 9. It appears to have a replacing or substituting power by which PO₄ is set free from the complex, as suggested by Demolon (2) and others.[3]

Other salts added with Na_2SiO_3 seem to act as they do with ammonium, as already indicated, increasing the solubility of PO₄ or not, independently of the presence of soluble silica.

Lowering of pH as by addition of an acid, or by other means, generally increases the solubility of PO₄ as the pH becomes lower, but there is much dif-

TABLE 9

Fixing power of soil 35 as affected by several reagents

100 gm. soil in 100 cc. solution + 15 mgm. PO₄

FILTRATE NO.	PO₄ FIXED WITH 0.1 N HCl				PO₄ FIXED WITH Na AS Na₂SO₄		
	0 cc.	5 cc.	10 cc.	20 cc.	10 mgm.	20 mgm.	40 mgm.
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	0.36	0.20	0.16	0.12	0.24	0.24	0.24
2	0.40	0.32	0.40	0.28	0.32	0.32	0.36
3	0.66	0.50	0.58	0.40	0.50	0.58	0.62

FILTRATE NO.	PO₄ FIXED WITH NH₄ AS (NH₄)₂SO₄				PO₄ FIXED WITH Na₂SiO₃:5 H₂O		
	10 mgm.	20 mgm.	40 mgm.		50 mgm.*	100 mgm.*	100 mgm.†
	p.p.m.	*o.p.m.*	*p.p.m.*		*p.p.m.*	*p.p.m.*	*p.p.m.*
1	0.24	0.04	0.28		0.24	0.40	0.20
2	0.28	0.08	0.40		0.36	0.80	0.58
3	0.40		0.72		0.80	1.34	0.88

* 1 hour before filtering.   † 4 hours before filtering.

ference between different soils in this respect (see table 10). In some cases where the soil has high buffer power and very little acid is added so that pH is not much changed, the solubility of PO₄ may be decreased, through bringing into solution more Ca or Mn by the added H^+, as shown in table 10, soils 35 and 64. Teakle (20, p. 152) and Fudge (6) report the same thing.

Effect of replacement of Ca^{++} and Mg^{++} by H^+, K^+, or NH_4^+.—It was thought

[3] Since the PO₄ in these solutions was determined by the molybdenum blue method which produces a similar blue color with silica, it may be thought that the increase in PO₄ found was really due to silica. This appears improbable for the following reason. Never more than 25 mgm. $Na_2SiO_3 \cdot 5$ H₂O was added to any one solution in which PO₄ was estimated by color. To evaluate the effect of the SiO₃, 100 mgm. was treated as for determination of PO₄. The color was equal to that produced by 0.004 mgm. PO₄, so that the effect of 25 mgm. $Na_2SiO_3 \cdot 5$ H₂O was negligible.

that removal of easily soluble Ca and other ions causing fixation, by washing the soil with water, would decrease its fixing power. The first percolate from the previously washed soil contains much more PO_4 than that from the unwashed soil, indicating much less fixation by the washed soil. But after the first percolate, the situation is reversed; the unwashed soil gave up more PO_4

TABLE 10

Effect of increasing concentration of hydrogen ion on pH and PO_4, dissolved

(50 gm. soil + PO_4 + H_2SO_4 in 100 cc. equilibrium extracts)

SOIL	PO₄ ADDED	0.1 N H₂SO₄	pH	PO₄ IN EXTRACT
	mgm.	*cc.*		*p.p.m.*
1c	0	0	7.2	0.20
		5	6.2	0.40
		10	5.5	0.80
		20	4.5	1.67
38	0	0	6.7	0.20
		5	6.2	0.20
		10	5.5	0.29
		20	4.5	0.57
	2	0	7.0	0.44
		5	6.0	0.44
		10	5.5	0.50
		20	4.6	1.00
64	0	0	7.0	0.04
		5	6.0	0.04
		10	5.5	0.08
		20	4.5	0.36
	2	0	7.0	0.20
		5	6.0	0.20
		10	5.4	0.29
		20	4.5	0.67
35	0	0	5.5	0.03
		5	5.0	0.03
		10	4.5	0.06
		20	4.3	0.06
	8	0	5.5	0.40
		5	5.0	0.29
		10	4.5	0.20
		20	4.3	0.20

to the wash water than did the washed soil, so that the total PO_4 in four percolates from the unwashed soil is greater than from the washed soil in the case of soil 35, and about the same with soil 64.

Replacement of the high fixing cations, Ca and Mg, by H or K was expected to reduce the fixing power of the soil, and this seemed to be true, but on account

of deflocculation of the soil, the results are in question. In one experiment all the other bases of four soils were replaced by leaching with ammonium acetate, which was then washed out with methanol, and the fixing power of the treated soil was determined. So much humus dissolved that it was necessary to remove it by ignition before estimating PO_4 in the extracts. The PO_4 found was much more than would be obtained from the untreated soils to which the same amount of PO_4 had been added. But whether the increased PO_4 was from the extracted humus or was due to decreased fixation could not be determined. Replacement of all other cations by H_2SO_4 was tried, and here the deflocculation of the treated soil was so great that the effect on solubility of PO_4 could not be determined. All attempts to determine the fixing power of soils that had been deprived of their usual cations failed. However, the effect of the removal of soluble Ca from the soil in increasing the solubility of the PO_4 is shown by the following experiment. Samples of surface soil were taken from the same spot in a garden before and after rain. The analysis gave these results:

Date of Sampling	p.p.m. in 1:1 water extracts		
	PO₄	Ca	SO₄
October 15, after long dry time	2.2	50	80
November 3, after 3 inches rain	2.4	40	50
January 6, after 2 inches rain	2.6	35	40
March 3, after 2 inches rain	2.7	35	30

It is evident that downward leaching of the Ca, etc., had reduced the concentration of cations so that more PO_4 could dissolve in the soil solution.

Repeated wetting, followed by drying, is sometimes supposed to render soil PO_4 more insoluble. Results of an experiment to test this theory are given in table 11. The soils were mixed with $CaH_4(PO_4)_2$ and water enough to make them very muddy, then dried in a thin layer in open pans in the air. When dry, they were pulverized, mixed, and again wetted and dried. Finally the PO_4 in the dry soils was determined in the Truog extract and in 1/100 and 1/1 water extracts.

With soils 1c and 95, of relatively low fixing power, there was little change after the first drying. Soils 35 and 77 have greater fixing power, and in them repeated wetting and drying seem to have decreased the water-soluble PO_4.

The fixing power of a number of materials other than soil is recorded in table 12. Many substances, organic and inorganic, are able to fix PO_4; how, is not clear. Permutite, presumably a sodium zeolite without Ca, has very high fixing power; perhaps an exchange of SiO_3 for PO_4 takes place. Washed beet pulp, nearly pure organic, seems to have a negative fixing power. When dry, it takes up water faster than the PO_4 dissolved in the water so that the concentration of PO_4 in the remaining water is increased.

Relation of PO_4 to Fe and Ca dissolved by dilute H_2SO_4.—The PO_4, Fe, and Ca extracted from four soils by digestion with dilute H_2SO_4 for three days is shown in table 13. When the solvent was 0.005 N acid, the PO_4 dissolved was more

TABLE 11

Effect of repeated wetting and drying on fixation of PO₄

SOIL	TIMES DRIED	PO₄ IN SOLUTION		
		Truog extract	Water extract	
			1/100	1/1
		p.p.m.	*p.p.m.*	*p.p.m.*
1c	1	3.3	0.88	1.44
	2	3.3	0.84	1.24
	3	3.3	0.80	1.24
35	1	1.44	1.00	1.00
	2	1.32	0.72	0.68
	3	1.60	0.72	0.56
77	1	1.76	1.08	1.76
	2	1.76	0.96	1.76
	3	1.76	0.96	1.32
95	1	1.32	0.80	8.80
	2	1.32	0.80	8.80
	3	1.32	0.80	8.80

TABLE 12

Fixation of PO₄ by various substances

SUBSTANCE	AMOUNT	PO₄	
		Added	Fixed
	gm.	*mgm.*	*mgm.*
Delta peat	20	2.0	0.886
German peat	10	1.0	0.500
Garden compost	20	1.0	0
Wood flour, spruce	10	1.0	0.42
Permutite	20	15.0	14.908
Kaolin	20	1.0	0.80
Diatomaceous earth	20	1.0	0
BaSO₄, very fine	20	1.0	0
Talc, powdered	20	1.0	0.4
Filter paper	10	1.0	0.2
Beet pulp (washed)	10	5.0	0
Talc	20*	0.12	0.06
Pumice	20*	0.12	0.048
Feldspar	20*	0.12	0
Mica	20*	4.00	3.76
Infusorial earth	20*	0.40	0.336

' Through 100-mesh sieve.

than equivalent to the Fe dissolved from soils 35, 59, and 64. But when the acid was 0.01 N, the iron dissolved was in all cases more than equivalent to the PO$_4$, and with stronger acid a still larger proportion of Fe was dissolved. Excess Ca was always dissolved. It is inferred that the PO$_4$ dissolved by the .005 N acid was probably present in the soil in combination with Ca. But the excess Fe dissolved by the stronger acids may indicate that most of the PO$_4$ in the soils was held in combination with iron. Heck (10) offers some information on this point. Dean (1) finds electrodialysis a useful means of obtaining information in regard to the phosphate compounds in soil. He

TABLE 13

PO$_4$, Fe and Ca in acid extracts of soils

2 gm. soil in 200 cc. solution, shaken 2 days

SOIL	NORMALITY OF H$_2$SO$_4$	AMOUNT DISSOLVED		
		PO$_4$	Fe	Ca
		m.e.	*m.e.*	*m.e.*
35 (pH 5.4)	0.005	0.012	0	....
	0.010	0.028	0.14	....
	0.050	0.120	5.3	......
	0.100	0.200	13.2	....
59 (pH 6.0)	0.005	0.001	0	0.3
	0.010	0.004	0.017	0.7
	0.050	0.025	0.190	1.0
	0.100	0.062	0.648	1.2
64 (pH 7.0)	0.005	0.028	0.014	3.0
	0.010	0.026	0.130	7.0
	0.050	0.106	2.140	25.0
	0.100	0.214	2.970	25.0
78 (pH 4.8)	0.005	0.008	0.024	0
	0.010	0.016	0.071	0
	0.050	0.034	0.324	0
	0.100	0.064	0.400	0

agrees with Heck. Iron phosphate complexes in soil are not well decomposed by this method, nor are the very insoluble basic phosphates of some calcareous soils. But the ordinary calcium phosphates of non-calcareous soils readily give up their PO$_4$ by electrodialysis.

Causes of fixation

Results given in the preceding section, "Phenomena of Fixation," indicate some of the causes. The effect of iron and aluminum colloids has been well dealt with by Gile (9) and others mentioned in the introduction, and was little considered in the present study.

The influence of pH is most important, since phosphates of Ca and Mg become easily soluble below pH 7 whereas Fe and Al phosphates are not much dissolved above pH 3.   But colloids of Fe and Al may fix PO_4 at pH 3–8.

Above pH 8, they may hydrolyze, so that PO_4 becomes soluble in alkaline solutions though Fe does not.   Below pH 7 added Ca^{++} or Mg^{++} depresses solubility of PO_4 and above that makes it nearly insoluble.   Removal of Ca from the solution greatly increases solubility of PO_4 as shown by Teakle (20, p. 147). $CaCO_3$ and $MgCO_3$ raise pH and increase Ca^{++} and Mg^{++} so that PO_4 is only slightly soluble in their presence.   The finer the particles of soil the greater is their fixing power, as shown in table 7.

The composition of the complex particles of the soil must greatly influence fixation.   If they are inert like quartz, they will have little fixing power, but reactive substances like kaolin or mica may have considerable effect.   Fixation by a number of substances, both organic and mineral, is shown in table 12. It appears that diatomaceous earth—which is mostly silica—, feldspar, and barium sulfate, have no fixing power.   Talc, mica, kaolin, and an impure infusorial earth have considerable fixing power.   Most fixation is probably the result of chemical action, yet some must be due to physical forces located in or on the surfaces of the particles of such substances as talc, kaolin, peat, and filter paper (see table 12).   Whether 90 per cent of fixation is due to chemical action, as indicated by Teakle (20, p. 160), is questionable.   Doughty (3) has shown that the organic matter of peat has much fixing power.   The fixing power of colloidal iron and aluminum compounds has been found great by Gile (9), and both he and Scarseth and Tidmore (18, 141) found that the fixing power of soil colloids varies inversely as the ratio $\dfrac{SiO_2}{R_2O_3}$ of the colloid.

Soil 59 used in the present study has the highest fixing power of any studied and it has the greatest proportion of hydrated iron oxide.

Control of fixation

In a practical way, it is very desirable to know how to treat a soil to increase or decrease fixation.

Increase of fixation is caused by raising the pH; by adding $CaCO_3$ or other carbonates or solutions of Ca, Mg, and Fe; and by increasing colloidal matter, such as by deflocculating.

Decrease of fixation is brought about by reversing these processes and also by adding ammonium or sodium salts or silica solutions.

Probably one of the best methods for getting soluble phosphorous deeply into the soil is to add it in some easily decomposed organic compound or in a colloidal complex not subject to fixation and wash it down by flooding.

Changes in fixation caused by addition of several substances is summarized in table 14.

Placement of phosphate in the soil in the most favorable location for absorption by the roots may considerably modify the effect of fixation.   Hockensmith

(11) and his associates found that favorable placement of the fertilizer largely increased absorption by plants. Gericke (8) made a considerable study of this factor and found that concentration of the added superphosphate in a zone of 5 cm. thickness at 20–25 cm. depth gave best results with cereals, wheat, oats, rye, barley. This increased the yield 13–27 per cent over that obtained when the fertilizer was uniformly distributed through the upper 30 cm. of soil. The chief difficulty is to place the phosphate in the most favorable location, which is largely dependent on the fixing power of the soil.

Midgley (16) and others have found it advantageous, in the case of high fixing soils, to apply the fertilizer in relatively large sized particles, in which

TABLE 14

Increase or decrease of fixing power caused by addition of various substances

50 gm. soil in 100 cc. solutions, shaken 1 hour, filtered on Büchner, and washed with 10 p.p.m. Ca and 5 p.p.m. Mg

SOIL	FILTRATE NO.	PO₄ ADDED	PO₄ IN FILTRATES AFTER ADDITION TO SOIL OF				
			H₂O only	CaCO₃ 1 gm. + PO₄	CaSO₄ 0.23 gm. + PO₄	Na₂SiO₃ 0.2 gm. + PO₄	(NH₄)₂SO₄ 0.05 gm. + PO₄
		mgm.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
64	1	10	0.06	4.0	2.5	4.0	...
	2		0.04	4.4	4.0	6.6	...
77	1	15	0.06	4.0	....	5.7	...
	2		0.04	8.8	....	20.0	...
95	1	1	1.24	8.8	....	8.0	...
	2		1.16	6.6	....	10.0	...
35	1	15	0.06	0.85	1.35	2.0	2.1
	2		0.04	0.65	1.10	5.0	2.7
59	1	25	0	0.72	0.66	0.50	...
	2		0	0.66	0.62	1.60	...
	3		0	0.62	0.58	2.66	...

the PO₄ remains in a soluble condition some time, instead of in fine particles, which are rapidly fixed. Scarseth and Tidmore (18, p. 161) point out the advantage of using pellets instead of mixing throughout the soil.

Deep placement of phosphate is desirable for fruit trees, but has not yet been successfully and economically accomplished.

SUMMARY

A review of the papers of ten investigations indicates that fixation of phosphate in soil in order of decreasing importance is most influenced by pH, Ca⁺⁺, and ratio $\frac{SiO_2}{R_2O_3}$ in the soil colloids.

A simple method is described whereby the relative fixing power of soils may be estimated and given appropriate numerical representation.

"Fixing power" is defined as the number of milligrams PO_4 per kilogram soil that must be added so that a 1:1 water extract will contain 1 p.p.m. PO_4.

The influence of time, ratio $\dfrac{\text{soil}}{\text{water}}$, size and composition of soil particles, and the effect of addition to the soil of H^+, Ca^{++}, Mg^{++}, K^+, Na^+, NH^+, soluble silica, and $CaCO_3$ are studied and the results discussed.

Many other substances than soil are found to have the ability to fix soluble phosphate.

Means for modifying fixation are: to increase fixation, raise pH and add Ca^{++}, $CaCO_3$, and soil colloids; to decrease fixation, do the opposite, also add Na^+, NH_4^+, soluble silica, organic colloids, organic compounds of phosphorus, employ special methods of placement, and use pellets instead of fine particles of the phosphate fertilizer.

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THE DETERMINATION OF ABSORBED BASES BY BOILING WITH AMMONIUM CHLORIDE AND THE UTILITY OF THE PROCEDURE IN RELATED SOIL INVESTIGATIONS

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In the determination of exchangeable bases in soils by methods such as those of Gedroiz (3), Hissink (4), and Kelley and Brown (5), cold ammonium chloride extractions have been used extensively. This salt is an effective exchange agent, but its advantages are offset by its solvent action upon calcium carbonate. Recently Di Gléria (2) showed that boiling ammonium chloride effected stoichemetrical decomposition of precipitated carbonates. He proposed that this treatment be used to eliminate soil carbonates in base-exchange studies and that the ammonia found in 200 ml. of distillates from boiling ammonium chloride suspensions be used to compute the carbonates. Di Gléria concluded that absorbed bases effect no liberation of ammonia from the boiling ammonium chloride solution. His proposal for the determination of soil carbonates was therefore based on the premise that the ammonia evolved is due solely to the reaction indicated by the equation:

$$CaCO_3 + 2NH_4Cl \rightarrow CaCl_2 + (NH_4)_2CO_3$$
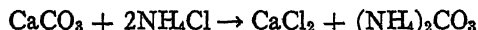
Because of the possible adaptation of this principle to certain absorption and base-exchange investigations at this station, it seemed advisable to study the solvent action of boiling ammonium chloride upon unusual carbonate and absorbed residues from different calcic and magnesic additions to certain soils, especially several of known lysimeter history. The results of the study and the several procedures developed therefrom are given in this paper.

THE DECOMPOSITION OF LIMESTONE AND OF DOLOMITE OF VARYING DEGREES OF FINENESS IN BOILING SOLUTIONS OF AMMONIUM CHLORIDE

The first distillations were made to insure complete disintegration of substantial occurrences of both limestone and dolomite by the boiling with ammonium chloride. In studying this point, together with the speed of decomposition, 0.5-gm. charges of limestone and of dolomite were used without soil. This represents a 10-gm. charge of a soil containing 5 per cent of $CaCO_3$. The factor of degree of fineness was introduced by the use of four separates of lime-

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[1] The analytical procedure was developed by the senior author. The junior author is responsible for the presentation.
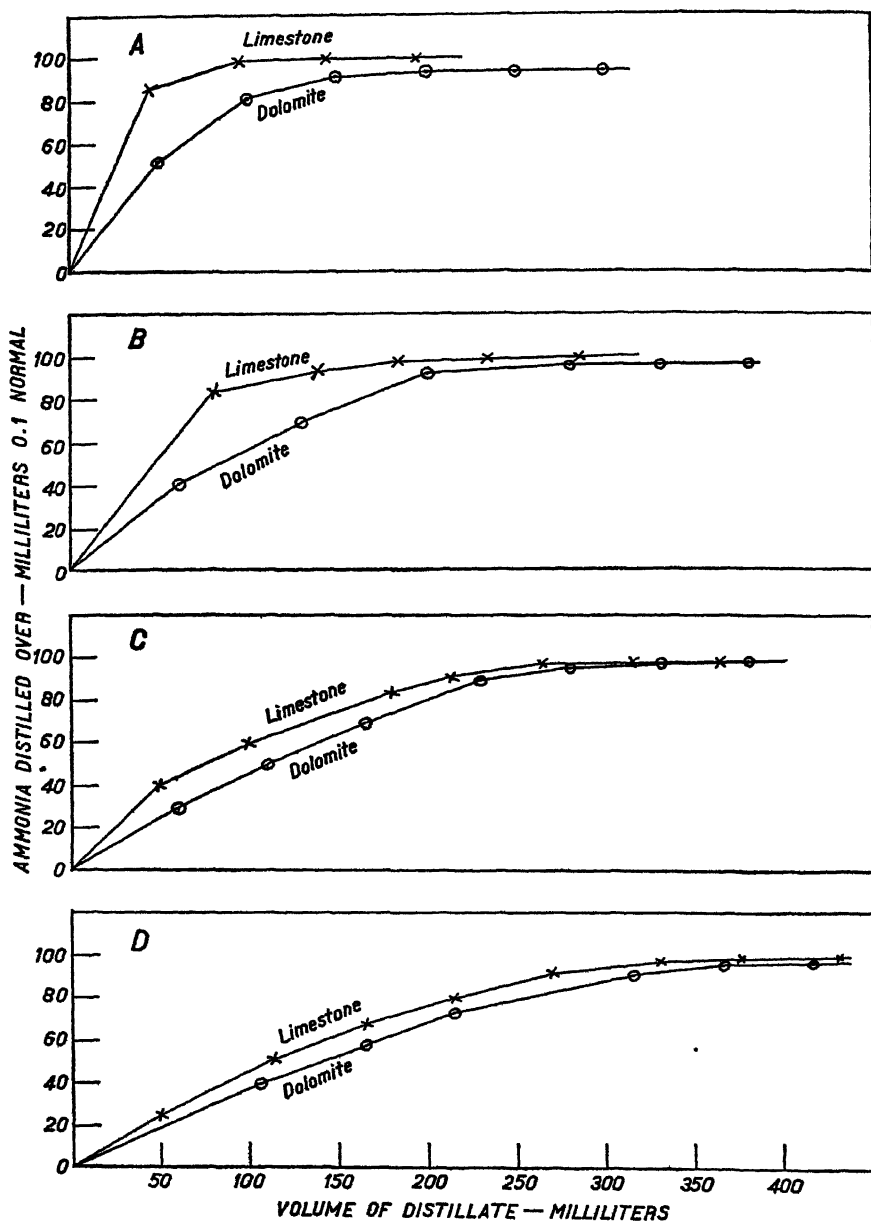
FIG. 1. DISINTEGRATION OF LIMESTONE AND OF DOLOMITE BY BOILING NORMAL SOLU
TIONS OF AMMONIUM CHLORIDE, AS DETERMINED BY AMMONIA DISTILLATIONS

A—Separates through 325-mesh.
B—Separates through 200-mesh, but stopped on 325-mesh.
C—Separates through 100-mesh, but stopped on 200-mesh.
D—Separates through 60-mesh, but stopped on 100-mesh.

stone and of dolomite. To each charge were added 100 ml. of a neutral normal solution of ammonium chloride and 200 ml. of water in a distillation flask. The distillation apparatus used throughout the present work was the all-glass Pyrex unit 1370. The mixtures were boiled and the distillates delivered into a standardized acid. The ammonia contents of successive measured fractions were determined at definite intervals, and these data were used to construct the four curves of figure 1. The evolutions of ammonia were plotted against the distillate volumes. The volumes can be translated into time by assigning 200 ml. per hour as the average rate of distillation.

Curve A shows that the limestone decomposition was much more rapid than that found for dolomite. Boiling for 30 minutes was sufficient to effect complete decomposition of the 0.5-gm. charge of 325-mesh limestone, whereas 1 hour was required for complete disintegration of the corresponding charge of dolomite.

The speed of the decomposition of the 200-mesh charges is shown in curve B. The initial speed found for the limestone was again much greater than that found for the dolomite. The complete decomposition of the sample of 200-mesh limestone required about 1 hour, whereas the dolomite sample required 1 hour and 15 minutes. The decompositions shown for the 200-mesh separates were considerably less rapid than those shown for the 325-mesh separates in curve A.

The 100-mesh data are plotted in curve C. The decomposition curves found for the 100-mesh separates of the two limestones are more concordant than those shown for the finer meshes, $1\frac{1}{2}$ hours being requisite for the complete decomposition of either the limestone or the dolomite.

The 60–100-mesh data are shown in curve D. The speed of disintegration of the less soluble dolomite approximated that found for limestone, the ammonia-liberation curves of the two separates of this mesh being almost parallel. Approximately 2 hours was required for the complete decomposition of the 60–100-mesh limestone and the dolomite in the boiling solution of ammonium chloride.

Hence, if 60-mesh separates are assumed to be representative of the limestone residues in field soils, or if the soil samples are reduced to that fineness, boiling for 2 hours with a normal NH_4Cl solution should effect complete decomposition of a 5 per cent calcium carbonate content of a 10-gm. charge of soil.

The relationship between the speed of decomposition of limestone and that of dolomite outside of the soil system, as influenced by the size of particles, is interesting in relation to previous observations as to the soil's capacity to effect decomposition of economic additions of limestone and dolomite separates. The carbonate residues from the coarser separates of dolomite were greatly in excess of those found for corresponding limestone additions, but this difference disappeared when separates of 80–200-mesh were used (6).

THE TENDENCY OF SOIL TO RETAIN THE NH$_4$ IONS DERIVED FROM BOILING
AMMONIUM CHLORIDE

The next step was to determine whether the soil exerted any retentive effect upon the ammonia released from boiling ammonium chloride. In the study of this factor a very acid sandy loam, practically devoid of exchangeable bases, and a stiff clay acid subsoil that contained 5.8 m.e. of exchangeable calcium were used. Mixtures of 10-gm. charges of soil, 100 ml. of normal ammonium chloride, and 200 ml. of water were boiled, and the ammonia content of the first 200-ml. distillate was determined in 50-ml. fractions. Similar distillates were obtained from the same soils supplemented by precipitated CaCO$_3$ and also from the NH$_4$Cl blank.

The results given in table 1 show that neutral ammonium chloride yielded appreciable quantities of NH$_4$OH when boiled alone for 1 hour, the period required to obtain four 50-cc. distillates. Based on a 10-gm. charge, the

TABLE 1

Tendency of soil to retain a part of the NH$_4$ radical derived from ammonium chloride on boiling, with and without CaCO$_3$ additions

SYSTEM	0.1 N NH$_4$OH LIBERATED				
	1st 50 ml.	2nd 50 ml.	3rd 50 ml.	4th 50 ml.	Total 200 ml.
	ml.	*ml.*	*ml.*	*ml.*	*ml.*
NH$_4$Cl + H$_2$O only (pH 7.0).................	1.80	0.60	0.30	0.15	2.85
NH$_4$Cl + sandy soil.........................	0.65	0.60	0.60	0.60	2.45
NH$_4$Cl + subsoil...........................	1.30	0.70	0.60	0.60	3.20
NH$_4$Cl + soil + 0.5 gm. CaCO$_3$...............	94.00	1.80	0.60	0.60	97.00
NH$_4$Cl + subsoil + 0.2 gm. CaCO$_3$...........	37.00	2.00	0.80	0.70	40.50

ammonia of the total distillate would be equivalent to 2.85 m.e. per 100 gm. of soil. When to this system was added 10 gm. of the very acid sandy loam, a small decrease in the ammonia content of the 200-ml. distillate was found. This acid soil, effecting pH decrease through exchange of its H for the NH$_4$ of the solution, apparently exerted a buffering effect on the rate of ammonia-release, making it nearly constant for the four fractions, whereas nearly two-thirds of the ammonia-yield from the neutral blank was in the first fraction. The acid clay subsoil also showed a buffering effect on the first fraction of the distillate.

The addition of 0.5 gm. of CaCO$_3$ to the boiled suspension of the acid soil yielded the equivalent of 97 ml. of 0.1 N NH$_4$OH, and when corrected for the soil blank, this represents a deficiency of 5.45 ml. of 0.1 normal ammonia. The addition of 0.2 gm. of CaCO$_3$ to the subsoil gave a 2.70-ml. deficiency in the ammonia yield, when corrected for the subsoil blank. The smaller deficiency from the clay soil reflects the balancing effects of the exchangeable calcium. The data of table 1 therefore demonstrate that a part of the ammonia that

was derived from the boiling solution of ammonium chloride was retained by the soil, either alone or with $CaCO_3$, and that this retentive tendency was diminished by exchangeable bases.

THE EFFECT OF ABSORBED[2] CALCIUM AND MAGNESIUM ON THE RELEASE OF AMMONIA FROM AMMONIUM CHLORIDE

To determine more specifically this influence on the release of ammonia from ammonium chloride, three soils of varying content of absorbed calcium and magnesium, with and without concomitant carbonates, were used. Each 10-gm. charge was introduced into a 400-ml. volume that included 100 ml. of N NH_4Cl, and a 200-ml. distillate was delivered into standardized acid. Two additional 50-ml. fractions were collected to determine the ammonia

TABLE 2

The capacity of the absorbed calcium and magnesium of certain humid soils to liberate ammonia from a boiling solution of ammonium chloride*

SOIL NO.	PREVIOUS TREATMENT	RESIDUAL CARBONATE CONTENT	ABSORBED Ca + Mg IN EXTRACT	AMMONIA IN DISTILLATES				
				1st 200 ml.	5th 50 ml.	6th 50 ml.	Total 300 ml.	Excess over carbonate value
		m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
1. Sandy loam.....	Limestone	0.8	10.5	10.45	0.80	0.70	11.95	11.15
2. Silt loam........	Limestone	1.6	12.5	10.40	0.70	0.70	11.80	10.20
3. Silt loam.......	Burnt lime	44.2	20.3	58.40	1.30	1.00	60.70	16.50
4. Silt loam.......	Limestone	59.6	20.4	73.50	1.50	1.00	76.00	16.40
5. Clay subsoil.....	Limestone	33.6	12.2	40.50	0.60	0.50	41.60	8.00
6. Clay loam.......	None	0	19.5	4.50	0.50	0.40	5.40	5.40
7. Clay loam.......	Magnesium carbonate†	21.8	47.0	63.90	2.20	2.10	68.20	46.40
8. Clay loam......	Magnesium carbonate†	2.0	12.0	14.40	1.00	1.00	14.40	14.40

* Expressed as milliequivalents per 100 gm. of soil.
† Normal crystalline, $MgCO_3 \cdot 3H_2O$.

released during the final stage of distillation. The data thus obtained are given in table 2. It is evident that the ammonia liberated from the ammonium chloride is not due solely to the carbonates of calcium and magnesium. The absorbed calcium and magnesium effected a liberation of ammonia that is evidenced when the sixth column of table 2 is compared with the two preceding columns of the same table. These data indicate that the reactions involved in the liberation of ammonia from a boiling ammonium chloride solution and a soil are represented by the following equations:

[2] The expression "absorbed" will be used to connote the bases absorbed and those exchangeable (8).

$$1. \quad CaCO_3 + 2NH_4Cl \rightleftarrows CaCl_2 + (NH_4)_2CO_3$$
$$2a. \quad CaX + 2NH_4Cl \rightleftarrows (NH_4)_2 \, X + CaCl_2$$
$$b. \quad (NH_4)_2 \, X + 2H_2O \rightleftarrows H_2X + 2NH_4OH$$
$$3a. \quad MgX + 2NH_4Cl \rightleftarrows (NH_4)_2X + MgCl_2$$
$$b. \quad (NH_4)_2X + 2H_2O \rightleftarrows H_2X + 2NH_4OH$$

The designations CaX, MgX, $(NH_4)_2X$, and H_2X represent the soil complexes that carry the respective cations.

In a comparison of the data in the last column with those in the fifth column it is evident that the second and third forward reactions do not go to completion, and that varying quantities of the NH_4 ion are retained, depending upon the specific nature of the soil complex. The reversal of the reaction is not due to the peculiarity of the absorbed ions of calcium and magnesium as against those derived from the $CaCO_3$ and $MgCO_3$ content of the soil, but rather to the specific ability of each soil to hold the liberated ammonia at the last

TABLE 3

Retention of ammonia by soils after distillation with ammonium chloride, and subsequent removal of the solute by alcohol washings

SOIL NO.[*]	PREVIOUS TREATMENT	RETAINED NH_4[†]	DISTILLATE NH_4 IN EXCESS OF CO_2	TOTAL NH_4 RECOVERED	ABSORBED Ca + Mg
		m.e.	*m.e.*	*m.e.*	*m.e.*
1. Sandy loam...............	Limestone	2.1	11.15	13.25	10.5
2. Silt loam...................	Limestone	5.5	10.20	15.70	12.5
6. Clay loam..................	None	15.7	5.40	21.20	19.5

[*] Those of table 2.
[†] And yielded by subsequent distillation with MgO.

experimental stage of the distillation. When in contact with NH_4Cl, the soil will exchange its several basic ions, including H ions, for NH_4 ions; and the equations 2b and 3b indicate what transpires when a soil is present in the system with NH_4Cl solution.

This point was demonstrated by another experiment, in which several soils were distilled with NH_4Cl solution. Each residue was filtered and washed with alcohol to remove solute NH_4Cl. The NH_4 retained by each soil was then determined by adding MgO and distilling. The results thus obtained and shown in table 3 confirm the conclusion that any disparity between the ammonia content of distillates and the ammonia equivalence of total absorbed bases is not due to incomplete reaction between the NH_4Cl and the absorbed bases, but rather to the capacity of the soil complex to fix the literated ammonium ion, the extent of fixation varying with the nature of the soil complex. The total yield of ammonia, the sum of that released and carried over in the distillate and that recovered from the soil residue, is somewhat in excess of the total

of bases displaced. This is accounted for in part by the ammonia given off by the NH_4Cl blank of table 1, a factor not considered in the foregoing discussion.

The data of tables 2 and 3 demonstrate that when a soil rich in carbonates is boiled with a solution of ammonium chloride, the resultant liberation of ammonia is due, not only to carbonates, but also to *the absorbed calcium and magnesium.* This is in direct contradiction to Di Gléria's conclusion that the ammonia-liberation in the soil-NH_4Cl system is due entirely to the carbonate content of the soil, and expressed solely by the equation,

$$CaCO_3 + 2NH_4Cl \rightleftarrows CaCl_2 + (NH_4)_2CO_3 \rightarrow (2NH_3 + H_2O + CO_2)$$

The data of table 3 further indicate that the absorbed and carbonate bases were completely displaced by the NH_4 ions within the distillation periods. This is evidenced by the fact that the disparities between the ammonia contents of the distillates and the joint equivalences of the extracted carbonates and absorbed bases were fully accounted for by the subsequent recoveries of ammonia from the washed soil residues.

THE SOLVENT ACTION OF BOILING AMMONIUM CHLORIDE ON THE SOIL COMPLEX

Since complete extraction of the bases present in both the absorbed and the carbonate forms was effected by the boiling digestion and supplementary leaching, the solvent action of the boiling ammonium chloride solution was greater than that found for cold dilute acid extractions in related studies (8). But the removal of the same bases by means of a dilute acid or an acid of moderate concentration would necessitate the application of heat and this would cause considerable quantities of iron, aluminum, and silica to go into solution during the extraction process. The question then arises as to the solvent action of the boiling ammonium chloride extraction upon the amphoteric components of the soil complex. To elucidate this point, the combined filtrates and washings from the ammonium chloride digestion were neutralized with ammonia and digested to flocculate the precipitates of the iron and aluminum hydroxides, which were ignited and weighed jointly as Fe_2O_3 and Al_2O_3. Two saline Colorado soils were included in this comparison. These two soils were rich in carbonates and were heavily impregnated with salts of Ca, Mg, Na, and K, as will be seen by reference to their analyses (9). The quantities of Fe_2O_3, Al_2O_3, and SiO_2 dissolved from the soils by boiling in an ammonium chloride solution for $1\frac{1}{2}$ hours were surprisingly small for all soils, as shown by the data of table 4. The solvent action exerted by the boiling ammonium chloride on sesquioxides was only a small fraction of that previously found for cold extractions with 0.05 N HCl. In a related study it was determined that a 1-liter leaching with 0.05 N HCl failed to extract all of the calcium from the limed soils of the Pennsylvania station plats, soils 3 and 4 of table 2; nevertheless, the average amount of $Fe_2O_3 + Al_2O_3$ removed by the dilute acid solution was 0.37 per cent of the soil charge.

It should be recorded that, at the conclusion of the distillations, the liquid phase of each soil-ammonia chloride system had a pH value ranging from 4.4 to 4.6, irrespective of the wide range of pH values that obtained at the beginning of the distillation. The initial pH values of the soils ranged between 4.4 and 10.4, whereas the original solution of ammonium chloride had a constant pH of 7.0. The low solubility of alumina in the boiling solution that ultimately attained and maintained a pH value close to 4.4 is ascribed to the stabilizing effect of the NH_4 ions absorptively held by the soil complex during the digestion.

Moreover, the boiling ammonium chloride extraction exerted no determinable solvent action upon the mineral forms of calcium and magnesium that are soluble only in strong HCl. The residue of one sample that had yielded 10 m.e. of exchangeable magnesium and still contained 80 m.e. soluble in HCl of 1.115 sp. gr., was subjected to an additional 1-hour digestion with boiling ammonium chloride and the subsequent washing. The *second* boiling ammo-

TABLE 4

The solvent action of boiling normal ammonium chloride solution upon soil content of Fe_2O_3 and Al_2O_3 and SiO_2

SOILS	$Fe_2O_3 + Al_2O_3$ DISSOLVED FROM 10-GM. CHARGES	SiO_2
	gm.	*gm.*
Sandy loam, high in acid soluble Al_2O_3..........	0.0046	None
Silt loam, previously limed....................	0.0016	None
Silt loam, previously limed....................	0.0009	None
Acid clay, previously limed....................	0.0025	None
Saline dolomitic Colorado sandy loam*.........	0.0200	0.09
Saline calcareous Colorado clay†..............	0.0600	0.12

* Total solids extracted by ammonium chloride digestion 13.83 per cent; † 7.64 per cent.

nium chloride digestion failed to extract any further determinable quantity of magnesium. But when the same soil was subjected to four 16-hour agitations with cold 0.02 N HCl, a continuous yield of magnesium was obtained. Hence, the single digestion with boiling ammonium chloride gave full recovery of the exchangeable magnesium, whereas the solvent effect was still manifest in the fourth extraction with the dilute acid.

THE ADEQUACY OF A 250-ML. LEACHATE-VOLUME FOR THE RECOVERY OF CALCIUM AND MAGNESIUM AFTER BOILING AMMONIUM CHLORIDE EXTRACTIONS

It seemed that the volume of ammonium chloride used in the Büchner washings could be reduced below the larger amounts used ordinarily in gravitational leachings. An untreated soil of high clay content, one of high magnesium content, and two soils of high calcium content were therefore used to determine the adequacy of 250 ml. for the digestion and washings. The soils

were all subjected to the preliminary treatment of NH_4Cl-digestion and -distillation, the residues being transferred to filters and washed with ammonium chloride. In one series the NH_4Cl filtrate and washings were limited to a combined volume of 250 ml.; in the other series an additional 250-ml. leachate was obtained. The calcium and magnesium contents of the filtrates were determined and are given in table 5.

These data show that practically the same quantities of calcium and magnesium were removed by the uniform digestion-distillation and the leachings of 250 ml. and of 500 ml. It therefore seems certain that, after the distillation treatment with normal ammonium chloride, a total volume of 250 ml. of filtrate and leachate is sufficient for the complete extraction of both the absorbed and the carbonate forms of calcium and magnesium, when the transferred digestion volume does not exceed 100 ml., i.e., when the leachate-washings are as much as 150 ml.

TABLE 5

The adequacy of 250 ml. ammonium chloride leachates for the recovery of calcium and magnesium after boiling with ammonium chloride (e)*

SOIL	PREVIOUS TREATMENT	250-ML. LEACHATES			500-ML. LEACHATES		
		Ca	Mg	Ca + Mg	Ca	Mg	Ca + Mg
		m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
Clay soil..............	None	11.0	10.00	21.0	11.2	10.20	21.4
Clay subsoil..........	Limestone†	45.4	0.96	46.4	45.5	1.66	47.2
Clay loam............	Burnt lime‡	64.5	1.32	65.8	64.6	1.40	66.0
Clay loam............	$MgCO_3$§	4.6	70.70	75.3	....	.....	74.4‖

* The final volume of the digestion being 100 ml.
† Greenhouse incorporation, 3 weeks.
‡ Pennsylvania station plat 23.
‖ By digestion with 1.115 HCl and deduction of mineral components.
§ Crystalline normal carbonate, 4 years' exposure in lysimeters.

UTILITY OF BOILING AMMONIUM CHLORIDE EXTRACTIONS FOR THE DIRECT
DETERMINATION OF TOTAL SOLUBLE MATTER IN HIGHLY CALCAREOUS
AND SALINE SOILS

It seemed probable that the procedure could be utilized advantageously with the soils of certain lysimeter experiments in which unusually heavy additions of either limestone or magnesic materials had been made. Certain of these enriched soils are deemed by the authors to be more difficult than most natural soils, either residual or transported. The chemical analyses of these enriched soils could not be expressed on the basis of the original soil, so great was the mechanical dilution caused by the substantial unabsorbed fractions of the added carbonates. In previous attempts to draw comparisons a series of analyses, such as carbonate CO_2 and acid-soluble calcium, magnesium, iron, and alumina, had been made. These determinations were supplemented and

amplified by computations, but the results were not satisfactory. In those cases where additions of magnesium oxide or carbonate had been made, the variable extent of the transition of added oxide to carbonate and the uncertainty as to degree of hydration of the residual carbonate militated against such computations. For example, assume an attempt to determine the organic-matter changes induced over a long period by a heavy magnesic treatment where the carbon content of the control soil was 2.20 per cent and that of the magnesia-augmented soil was 2.0 per cent. The apparent loss of 0.2 per cent, or 10 per cent decrease, below the original organic carbon content of the soil would not be an actual loss, since the soil had been diluted by the heavy addition of magnesia. Again, if carbonate CO_2 is found to be 4.30 per cent, then on the basis of a postulated occurrence of $MgCO_3$, the residual carbonate would be 7.23 per cent of the mixture; on the basis of $MgCO_3 \cdot 3H_2O$ occurrence, the value would be 13.52 per cent of the mixture; on the basis of $3MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$ occurrence, the value would be 11.90 per cent of the mixture. Hence, in the computations for residual CO_2 in possible magnesium carbonate variables, the correction would cover a range between 7.23 per cent and 13.52 per cent of the enhanced weights of the mixtures of the original soil and magnesic additions. This is sufficient to vary the carbon computations between a minus value and a plus value, in one case an indication of a slight loss of carbon, in the other, a decided increase of from 2.16 to 2.31 per cent of carbon.

There was also a considerable absorption of base by the soil—magnesium, absorption greatly exceeding that of calcium—which called for acid-extraction and analysis and additional corrections. It therefore seemed that, for the purpose of any single analytical determination such as that for the organic carbon or that for the nitrogen of heavily limed lysimeter soils, the simple extraction with boiling ammonium chloride should assure comparable residues of a specific soil, limed and unlimed, as the basis for computations of analytical data.

To throw light on this point, the three lysimeter soils of table 6 were studied —an unlimed clay loam, the same loam treated 10 years previously with $CaCO_3$, and the same loam likewise treated with magnesium hydroxy-carbonate. A 10-gm. charge of each soil, oven-dry basis, was placed in a 600-ml. Pyrex beaker, and 100 ml. of N NH_4Cl was added, together with 200 ml. of water. The beaker was covered with a watchglass and heated to vigorous boiling over a Bunsen burner. For this acid soil, and also for all acid soils studied, it was found that the boiling need be continued only until 200 ml. of water is driven off. But for those soils that contained large residues of either lime or magnesia, an additional 100-ml. volume of water was added and boiled off. In all cases the volumes in the covered beakers were replenished to prevent diminution below 100 ml. Complete disintegrations were obtained by boiling for 1 hour after the vapor ceased to give indication of ammonia. This was tested by placing a strip of moistened neutral litmus paper in the lip of the beaker

and comparing the color of this paper with that of a moistened blank. For dolomitic soils the more sensitive brom cresol blue test, given later, should be used.

After cooling, each mixture was transferred to a 56-mm. Büchner with N NH_4Cl and washed therewith through a *hardened* filter to a volume of 250 ml.; the clear filtrate was analyzed later for Ca, Mg, Fe_2O_3, Al_2O_3, and Mn_3O_4. The residue from the ammonium chloride washings was washed with 95 per cent ethyl alcohol until free of Cl ions and sucked dry; the alcohol required usually amounted to 300–400 ml. The residue was then policed with water into a porcelain evaporation dish of slightly larger diameter than the outside diameter of the Büchner, evaporated to a small volume on a steam bath, transferred to a small weighed platinum dish where the evaporation was completed. The residue was then dried in an oven at 105°C. for 2 hours, cooled in a desiccator for 30 minutes, and weighed rapidly to obviate gain in moisture.

The detailed analytical results for the limed soil of table 6 are expressed in percentage of the extracted oven-dried residues, whereas the data for the control soil are given on its original basis. The total solvent action exerted on the soil by this boiling and extraction procedure is shown in the lower space of table 6.

In computing the analytical values found for CO_2, CaO, MgO, etc., it was necessary to postulate the degree of hydration and carbonation of the residual magnesia. The computations were made on the assumption that, under the partial CO_2 pressures that prevail in the soil system, the most stable composition of the residual magnesia is the form $3MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$. It was difficult to prove the correctness of this assumption, which was used in reporting the outgo of magnesium (7) and in a study of the state of the non-carbonate residues of magnesium by the use of dilute acid extractions (8). Further supporting evidence is now found, however, in the close agreement shown for the sum of the hypothetical combinations and the actual loss on extraction, as registered by the bottom line of table 6.

On the other hand, if it be postulated that the residual magnesium is in the form of $MgCO_3 \cdot 3H_2O$, then the computed sums of the several components dissolved would be 16.51 per cent of the soil residue, or 3.46 per cent more than the actual determination of 13.05 per cent. Hence, after the complete removal of the accumulated fixed bases and residual carbonates by a boiling solution of ammonium chloride and supplemental leachates, the weighed residue from the magnesia-treated soil was thus found to be comparable with the residue of its control soil that had been subjected to the same analytical manipulation.

The carbonate residues from the added calcium carbonate occur in the original chemical form, since here no question of carbonate hydration is involved; hence, the data of the fourth column of table 6 do not require the hypothetical assumptions used in the computations that were applied to the

magnesia treatments. The concordance between the sum of the dissolved components and the direct loss on extraction from the calcareous soil demonstrates that the boiling ammonium chloride extraction and leaching is an accurate technic for the joint extraction of the residual calcium carbonate and the exchangeable calcium content of heavily limed soils.

A point to be emphasized is that the data also show that the boiling ammonium chloride exerts a meager solvent action on the other components of a calcareous soil. This solvent action was materially less than that found by the use of any known method that is capable of extracting all exchangeable bases. From the foregoing data and discussions it is evident that the pre-

TABLE 6

The actual loss of weight of unlimed, $CaCO_3$-treated, and $MgCO_3$-treated soils by the ammonium-chloride digestion-extraction procedure, in comparison with the summation of the separate components removed

DISSOLVED COMPONENTS	UNLIMED SOIL	CaCO₃-TREATED SOIL	MgCO₃-TREATED SOIL*
	per cent	*per cent*	*per cent*
1. Total calcium, CaO	(0.064)	(9.520)	(0.220)
2. Total magnesium, MgO	(0.013)	(0.065)	(5.760)
3. Carbonate CO_2	0	7.010	4.300
4. CaO or† MgO equivalence of CO_2	0	8.920	5.250
5. Absorbed calcium, CaO	0.064	0.600	0.220
6. Absorbed magnesium, MgO	0.013	0.065	0.510
7. H_2O due to residual† $MgCO_3$	.....	.....	2.350
8. H_2O due to hydration of absorbed CaO and MgO	0.026	0.221	0.301
9. Mn_3O_4	0.092	0.092	0.092
10. $Fe_2O_3 + Al_2O_3$	0.015	0.015	0.015
11. Summation of unbracketed data	0.210	16.92	13.04
12. Loss on extraction	0.310	17.07	13.05
13. Deficiency by summation of separate components	0.100	0.15	0.01

* Additions of precipitated hydroxy carbonate.
† Computed on the basis of CO_2 content as in $3MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$.

scribed boiling of a soil in an ammonium chloride solution and attendant washing offers a rapid and accurate procedure for the extraction of *absorbed bases and their carbonates in one operation*, with nugatory effect upon the acidoid residue and minerals.

Fineness of Soil Samples.—In using the subsequent procedures, it is recommended that the entire sample of ordinary soils be ground to pass a 0.5-mm. sieve. For soils of high content of either limestone or dolomite, grind to pass

a 0.25-mm. sieve. This fineness is not essential for complete carbonate decomposition, but is intended to insure a homogeneous sample.

AMMONIUM DISTILLATION PROCEDURE FOR THE RAPID DETERMINATION OF TOTAL ABSORBED BASES,[3] REGARDLESS OF NEUTRAL SALTS

In this paper it has been shown that the ammonia liberated from the digestion of soils with ammonium chloride is due in part to the decomposition of carbonates in the soil and in part to the displacement of the absorbed bases, primarily calcium and magnesium. But it was also noted that the liberated ammonia was not in exact equivalence to the sum of the carbonates and the absorbed calcium and magnesium, because of the fact that varying quantities of ammonia withstood the boiling and were retained by the soil. This finding can be used advantageously to determine accurately the total quantities of absorbed bases, regardless of the presence of carbonates, without a preliminary aqueous extraction of salts—since these do not vitiate the results—and without direct determination of the several bases present in the NH_4Cl extract.

The technic is as follows: Introduce into a 500-ml. distillation flask (preferably Pyrex unit 1370), 10 gm. of soil, 100 ml. of neutral normal ammonium chloride, and 200 ml. of ammonia-free distilled water. Boil the suspension and collect the distillate in standardized acid until a volume of 200 ml. is obtained. For acid soils in general this volume of distillate is sufficient. For unknown soils and those of high carbonate content a larger distillate may be required. To insure that complete reaction and distillation have taken place, 100 ml. of water is added and recovered by distillation. Repeat this step until 100-ml. fractions show a titration value of 1 ml. or less of 0.1 normality, at which point the small evolution of ammonia is attributable solely to the exceedingly slow release of the NH_4 ions retained by the soil.

Transfer the suspension to a 56-mm. Büchner filter and wash with unaltered 95 per cent ethyl alcohol until free of ammonium chloride. Return the ammonium-chloride-free residue to a distillation flask, previously washed free of any ammonium salts; add an aqueous suspension of 0.5 gm. of MgO; and distill 200 ml. into 0.1 normal acid, 5–10 ml. being usually required. Combine this titration value with that obtained from the ammonium chloride distillation, and express the combined value as milliequivalents, each ml. of 0.1 normality ammonia-yield representing 1 m.e. per 100 gm. of soil. From this value subtract the separately determined carbonate-CO_2 value (12) and the ammonia recovered in the first 50-ml. distillate of the blank NH_4Cl solution to obtain the milliequivalents of total absorbed bases.

Note.—The ammonium chloride distillations should be carried out in apparatus not previously used for Kjeldahl distillations, unless particular precautions are taken to insure complete absence of alkali from flask, stopper, and also glass trap.

[3] Hissink's S value.

BOILING AMMONIUM CHLORIDE DIGESTIONS FOR THE DETERMINATION OF
ABSORBED AND EXCHANGEABLE CALCIUM, MAGNESIUM (8), SODIUM,
AND POTASSIUM, REGARDLESS OF CARBONATES

It has been recognized that the presence of $CaCO_3$ in a soil precludes the use of ammonium chloride in the determination of exchangeable calcium, because of the solvent action of the chloride on $CaCO_3$.   Hissink (4) therefore suggested that NaCl be used and that supplemental extraction with NH_4Cl be carried out to determine exchangeable magnesium, sodium, and potassium.   The latter procedure requires, of course, the precise elimination of calcium, just as though a quantitative determination of that base were to be made.   Recently, Chapman and Kelley (1) suggested the use of 0.2 N KCl in a 68 per cent alcoholic solution for the determination of exchangeable calcium in calcareous soils.   It is obvious that, as in the use of the Hissink method, a supplemental extraction with ammonium chloride is required for the determination of the exchangeable sodium and potassium.

The ammonium chloride digestion procedure is carried out as follows: Introduce a 10-gm. charge of soil into 100 ml. of normal ammonium chloride in a 600-ml. beaker; add 200 ml. of water; cover the beaker and boil the volume of the suspension to 100 ml.   Replace the vaporized water in 100-ml. fractions, as in the distillation procedure, until a moistened strip of *high-grade neutral* litmus paper shows no indication of ammonia when exposed to the vapors passing from the lip, a control moistened strip being used for comparison. After absence of NH_3 is indicated, boil off 200 ml. additional.   An effective digestion and end-point control is provided by the following brom cresol blue test.

The brom cresol blue test for indicating NH_4Cl-soil digestion end-point.—Because of the variable quality of the litmus paper on the market it was found desirable to introduce a more sensitive test to indicate the end-point of decomposition of soil carbonates and base replacement in the covered-beaker digestion procedure.   After tests with a number of widely divergent soils the following dependable end-point test was adopted.   Prepare a 0.04 per cent solution of brom cresol blue by grinding 0.040 gm. of the dye in an agate mortar with 25 ml. ethyl alcohol; dilute with water; add 5 ml. of 0.1 N HCl and make to 100 ml.   This gives a 0.04 per cent brom cresol blue solution in 0.0005 N HCl.   Dip a slender stirring rod into this indicator solution; remove hanging drops and apply the end of the moistened rod to a 15 x 30 mm. strip of heavy non-acid washed filter paper.   This should make a distinct yellow spot of about 7–8 mm. diameter surrounded by a pale blue ring.   With tweezers, hold the test paper to the spout of the covered beaker of the boiling NH_4Cl-soil suspension so that the steam strikes the yellow spot.   If the yellow spot fails to change completely to a solid blue within 6–8 seconds, the digestion is complete.   The filtered solution will then have a pH of 4.4 ±0.2.

Filter, wash with eight 20-ml. washings of ammonium chloride, and make to

volume for the determination of the desired bases. If the soil contained calcium carbonate, the calcium in that combination can be corrected for by subtraction of the calcium equivalence of the CO_2 separately determined by use of a deoxidant (12). A similar correction can be applied for carbonate occurrences of magnesium in soils previously treated with magnesic materials. If the soil is a transported soil and is known to contain dolomite, and a high carbonate occurrence is paralleled by unusually large quantities of dissolved magnesium, the magnesium determination can be corrected by assigning thereto one half of the carbonate equivalence, the other half being applied to the calcium. This last procedure is not ideal, since the 1 to 1 ratio is not a constant for dolomites, but affords an approach to the proximate determination of exchangeable calcium and magnesium in those soils that contain residues of undisintegrated dolomite.

BOILING AMMONIUM CHLORIDE DIGESTION PROCEDURE FOR THE DETERMINATION OF BASE-EXCHANGE CAPACITY, REGARDLESS OF CARBONATES

To assure the subsequent saturation with ammonium chloride that is required for determination of base-exchange capacity in calcareous soils, it is essential to effect complete displacement of absorbed bases and removal of carbonates. It has already been shown that this result is accomplished by the boiling digestion with ammonium chloride according to the following procedure:

Boil the suspension vigorously, as directed for determination of absorbed and exchangeable bases. Remove from source of heat, *cool*, add 15 ml. of 0.1 N NH_4OH[4] to the suspension while stirring, and allow to stand at room temperature at least 1 hour. Then wash the soil residue on a 56-mm. Büchner funnel with neutral normal ammonium chloride, taking care to police all of the soil from the beaker and the cover glass. Wash the soil residue on the filter 10 times with 20 ml. N NH_4Cl per washing. Continue to wash on the same filter with neutral ethyl alcohol until the washings show no trace of Cl ions, taking care to wash any NH_4Cl that may creep up to the edge of the funnel. Transfer the alcohol-washed soil and its filter to a distillation flask, add 0.5 gm. of MgO in aqueous suspension and distill off the absorbed NH_4. On the basis of a 10-gm. charge of soil, each ml. of 0.1 normal ammonia in the distillate represents 1 m.e. of base-exchange capacity per 100 gm. of soil.

BOILING AMMONIUM CHLORIDE PROCEDURE FOR THE PREPARATION OF SOIL FOR MECHANICAL ANALYSIS

The International Method for the Mechanical Analysis of Soils prescribes that the soil be subjected to a preliminary digestion with 0.2 N HCl to decompose carbonates. According to Russell (11), this preliminary treatment may cause a loss of as much as 2 to 3 per cent of dissolved sesquioxides and silica.

[4] Empirically determined as the optimal to effect speedy neutralization of the built-up absorbed hydrogen and to minimize volume of ammonium chloride required to attain a neutral leachate.

To eliminate this difficulty, we advocate that the soil be digested with a boiling normal solution of ammonium chloride, as prescribed by us in the beaker-digestion method for the determination of total dissolved bases. As pointed out, this preliminary digestion effectively eliminates all of the carbonates and neutral salts without appreciable effect upon the sesquioxides or the silica of the soil. The actual loss in weight suffered by the analytical charge through the solvent action of the ammonium chloride is readily determined: Subtract the weight of the alcohol-washed and dried soil residue from the initial moisture-free soil weight. The mechanical analysis can then be carried out on the modified charge.

SUMMARY AND CONCLUSIONS

A study was made of the activity of boiling neutral normal ammonium chloride solution upon soils rich in carbonates and of high absorbed calcium and magnesium content.

The speed and completeness of the disintegration of limestone and of dolomite of different degrees of fineness, as measured by liberated ammonia in the absence of soil, was determined in a preliminary study.

The ammonia-liberations from soil suspensions in boiling neutral ammonium chloride were found to be caused, not only by carbonates, but also by absorbed bases, which Di Gléria had concluded were not affected by the ammonium chloride.

The ammonia-liberations were found to be closely approximate to the joint values of the carbonates decomposed and the absorbed bases replaced; but the total of dissolved bases could not be expressed as the absolute equivalence of the ammonia distilled from the ammonium chloride, because of the variation that soils show in their specific capacities to retain NH_4 ions. Absolute equivalence was attained, however, when the NH_4 held by the alcohol-washed and NH_4Cl-free soil was liberated by boiling with MgO and the ammonia so recovered was added to that obtained in the distillation with neutral normal ammonium chloride.

It was found that boiling ammonium chloride affected *complete* disintegration of the several types of carbonate and *complete* replacement of absorbed and exchangeable bases.

The method has the advantage of speed and requires only a small fraction of the ammonium chloride utilized by other methods.

In effecting complete removal of bases, the ammonium chloride exerted only a nugatory solvent action upon the sesquioxides and silica in both humid and saline soils.

Regardless of the initial soil reactions, pH 4—pH 10, the final pH values of the digestions were within the pH range of 4.4 to 4.6.

The alternative procedures: (*a*) quantitative distillation of ammonium and the supplementary CO_2 determinations, and (*b*) the beaker digestion for the

determination of the several bases, can be utilized in soil research with soil samples of such fineness as to insure uniform distribution of the carbonates:

A technic is prescribed for the determination of absorbed Ca, Mg, Na, and K by a single extraction, regardless of carbonates.

Total absorbed bases can be determined as the equivalent sums of the two values: (a) the ammonia evolved from the boiling soil suspension in neutral ammonium chloride and (b) the NH_4 ions retained by the soil and subsequently released by boiling with MgO, regardless of occurrences of soluble neutral salts.

Total bases can be determined in those soils that do not yield their absorbed magnesium content by protracted cold extractions with either dilute acids or cold ammonium chloride.

Experimental soils that are "diluted" with large residues of carbonates can be restored to a condition comparable to that of the same soil untreated, and thus admit comparisons otherwise not feasible.

Total base-exchange capacity can be determined subsequent to the boiling with ammonium chloride for the elimination of carbonates, by neutralization of the acid-reacting suspension and leaching of the soil residue with neutral ammonium chloride, removing the solute NH_4Cl, adding MgO, and distilling the absorbed NH_4.

The boiling ammonium chloride extraction can be used for the digestion of carbonate-impregnated soils prior to their mechanical analysis, in lieu of, and as a decided improvement upon, preliminary digestion with 0.2 N HCl as now prescribed in the international procedure.

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COLLOID CHEMICAL ASPECTS OF CLAY PAN FORMATION IN SOIL PROFILES

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Numerous soil types in the central part of the United States are characterized by heavy clay pans, i.e., accumulations of colloidal clay particles in the B-horizon of the soil profile. The pans form in loess under a cover of prairie or timber vegetation and are most conspicuous in areas of poor drainage. Typical clay pans such as those in Putnam silt loam cause serious handicaps to agricultural practices. In the springtime the surface water remains stagnant and seed-bed preparation is delayed. In general, plant root development is stunted and tree growing is almost impossible.

Figure 1 shows the distribution of clay particles less than 1μ (0.001 mm.) in diameter in a poorly drained and in a well-drained profile in the State of Illinois (5). It will be noticed that in certain parts of the soil column the clay particles make up 50 per cent of the soil weight.

Generally speaking, the origin of clay pans may be due to several causes: first, by deposition of colloids in water (geological process); secondly, by local formation of clay *in situ* by intensive weathering; and thirdly, by translocation and subsequent deposition of clay particles within the soil profile. This last type of pan we shall designate as *illuvial pan*, and all subsequent discussions will be restricted to this specific variety.

NATURE OF THE COLLOIDAL PARTICLES ISOLATED FROM ILLUVIAL CLAY PANS

Analyses of clay extracted from the various horizons of clay pan profiles indicate that the properties of the colloids vary but little, and one is forced to conclude that the clay particles migrate as a whole rather than in the form of colloidal SiO_2, Al_2O_3, and Fe_2O_3 with subsequent recombination. The particles isolated from the Putnam clay pan exhibit pronounced streaming double refraction, which proves that they are not of spherical shape. The X-ray patterns are very distinct and resemble those of montmorillonite.[1] The silica-alumina ratio fluctuates between 3.0 and 3.2, and that of the silica-sesquioxide ratio, between 2.5 and 2.8. The base-exchange capacity amounts to 60–70 m.e. per 100 gm. colloid, depending on the method of determination.

The constancy of the composition of the colloidal clay particles throughout

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[1] HOFMANN, U., AND ENDELL, K. X-ray analyses of Putnam clay. Unpublished data.

the profile justifies a study of their behavior in artificial sand columns under
controlled laboratory conditions.   Such investigations might be called experi-
mental soil profile formation, and the results should prove to be of value in
the interpretation of natural soil systems.
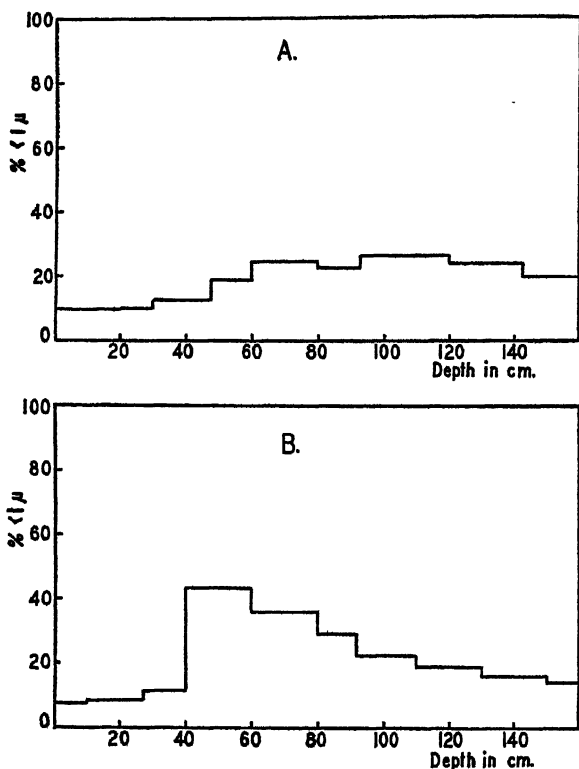
FIG. 1. ACCUMULATIONS OF CLAY PARTICLES LESS THAN 1μ IN DIAMETER IN VARIOUS
HORIZONS OF A WELL-DRAINED (A) AND A POORLY DRAINED (B) PROFILE

Ordinate indicates per cent clay per 100 gm. of soil

EXPERIMENTAL PROCEDURE

Since a detailed description of the experimental part is to be reported elsewhere, only the
most essential points will be mentioned here.   In principle, a series of artificial soil skeletons,
composed of quartz grains or spherical glass beads, was prepared, and clay suspensions (sols)
were passed through the beds.   The effects of various treatments on the velocity of pan forma-
tion in these artificial systems were recorded quantitatively.

Soil skeleton.—Carefully purified quartz particles of uniform size (table 1), were packed to a
height of 5–10 cm. into burettes of 12 mm. diameter.   A standard rate of flow of 5 cc. of dis-
tilled water in 23–24 seconds was arbitrarily selected, and the head necessary to give this
flow was determined empirically for each sand column.

Preparation of clay sols.—A sol was prepared by adding NaOH to an electrodialyzed Put-
nam clay at the rate of 60 m.e. of base per 100 gm. of colloid.   To insure equilibrium the
suspension was shaken at intervals for several weeks and then diluted to a 0.3 per cent sol.

Criterion of pan formation.—When properly treated Na-clay sols were allowed to percolate through the skeleton, the time required for 5 cc. to pass through varied in a regular manner. The change in the rate of flow was selected as the best measure of pan formation. Zero change indicates absence of clay accumulation, whereas a decreasing rate of flow corresponds to pan development. The results are reported as relative percolation velocities, calculated as follows: The time required to deliver the first 5 cc. of sol (t_1) was taken as unity. The time used by each successive aliquot (t_n) was then divided into that of the first 5 cc. $(t_1:t_n)$. Thus, when the time had doubled, the relative rate of flow or percolation velocity would be 0.5. In the graphs the fine dotted lines pass through the observational values, while the heavy lines represent the fitted trend.

INHERENT DISPERSION TENDENCY OF COLLOIDAL CLAY SYSTEMS

In absence of flocculation agencies the Putnam clay particles have a natural tendency to "disperse." Air-dry chunks of colloidal clay submerged in distilled water will hydrate and swell, and the particles will separate from one another. Although the Brownian movement is not intense enough to cause much diffusion and dispersion, it is important to note that the colloidal particles are free to glide easily along one another. Mere convection currents and minor disturbances, such as gentle shaking, cause the system to disperse sufficiently to form a temporary, or even a permanent, sol. Thus, Putnam clay in contact with water and not subjected to mechanical disturbance forms neither a sol nor a true gel, but a so-called *coacervate*, i.e. a gel-like system in which the particles possess individual mobility (2). This concept is essential for a clear understanding of the behavior of clay particles in soils.

When a fine clay sol is passed through beds of coarse quartz sand or glass beads, no pan formation occurs and, furthermore, the clay particles do not adhere to the skeleton except for negligible surface tension effects. Complete separation of quartz sand and colloidal clay by mere washing with distilled water is always possible. As a matter of fact, such a behavior would be expected on theoretical grounds, since both sand and clay are negatively charged and, consequently, repel each other.

Observations of the sort mentioned strongly suggest that under natural field conditions colloidal clay particles of the Putnam clay type tend to "disperse" and migrate with the water currents unless held back by some special mechanism.

THE SIEVE ACTION PRINCIPLE IN PAN FORMATION

In a soil profile the colloidal particles can migrate only in the pore channels of the soil skeleton. If the diameter of the smallest pore exceeds the greatest diameter of a clay particle, the latter will freely follow the percolating water and no tendency of pan formation will exist. In any other case partial or total accumulation of colloids is certain to occur. Generally it can be said that a soil skeleton can filter out or retain colloidal particles only if the pores themselves are of ultramicroscopic dimensions. Spherical quartz grains would need to possess radii of 0.2μ (order of magnitude), in other words, they would have to be clay particles also, though of somewhat larger size. Angular quartz

grains could be of macroscopic size, silt for instance, and still produce channels with occasional colloidal cross-sections. Evidently the *formation of clay pans by sieving out the colloids mechanically is restricted to very fine-textured soil skeletons.*

TABLE 1

Particle and pore size of soil skeletons (on the basis of spherical shape and close packing)

PARTICLE GROUP			DIAMETER OF PORES*
Conventional name	Taylor sieve designation	Diameter of grains	
	mesh	*mm.*	*mm.*
Beads............................	....	0.56–0.05	0.087
Coarse sand.......................	20– 40	0.83–0.39	0.129–0.054
Medium sand......................	40– 60	0.39–0.25	0.054–0.038
	60– 80	0.25–0.18	0.038–0.027
Fine sand.........................	80–100	0.18–0.15	0.027–0.023
	100–150	0.15–0.10	0.023–0.016
Very fine sand....................	150–300	0.10–0.05	0.016–0.008
Coarse silt.......................	....	0.05–0.02	0.008–0.003

*From Slichter (6).
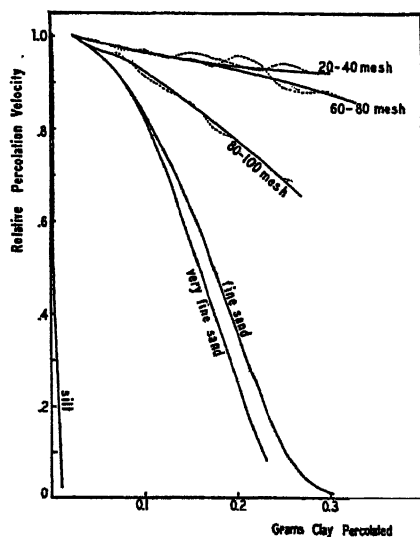
FIG. 2. THE SIEVE ACTION PRINCIPLE OF CLAY ACCUMULATION

Passage of a heterogeneous Na-clay sol through columns of coarse and fine quartz grains

Figure 2 illustrates instructively the sieve action principle of pan formation. Na-clay sols were passed through various sand beds, each consisting of quartz particles of different, but uniform, size. It is clearly seen that the smaller the average diameter of the grain the more rapid is the pan formation as indicated

by the reduction of the percolation velocity of the sol. Sand of 20–40 mesh caliber acts very slowly, whereas silt appears to be very effective. In order to explain fully the sequence of the curves it should be pointed out that the sol used contained, not only colloidal particles, but also larger ones. In fact, occasionally aggregates of microscopic size were found floating in the sol (bacterial colonies). The coarse sand is able to retain the large aggregates, and the rate of flow is slightly reduced. As the size of the quartz grains decreases, coarse clay particles are caught, which help to reduce the effective cross-section of the pores until the latter reach colloidal dimensions and almost stop the passage of the sol.

Besides using a specific clay sol and a variety of sand beds we percolated various clay sols through beds of equal particle size. For this purpose the original sol was fractionated by filtering it with a column of 60–80 mesh sand,

TABLE 2

Effect of mechanical fractionation of a clay sol on the velocity of pan formation in a fine sand bed

SUSPENSION PERCOLATED	RELATIVE PERCOLATION VELOCITY	
	Original sol	Finer fraction
First 5 cc. sol	1.000	1.000
Second aliquot	0.958	0.995
Third aliquot	0.914	0.995
Fourth aliquot	0.835	0.989
Fifth aliquot	0.742	0.966
Sixth aliquot	0.635	0.976
Seventh aliquot	0.509	0.953
Eighth aliquot	0.346	0.966
Ninth aliquot	0.238	0.961
Tenth aliquot	0.107	0.963

which removed the coarse particle group (microörganisms). Subsequently, both the original sol and the refined part (condensed to a 0.3 per cent suspension) were subjected to pan formation processes in beds of fine sand. According to table 2 the resulting differences are very great. The original sol develops a pan much more rapidly than the finer fraction.

In a natural soil both the skeleton and the mechanical composition of the clay group are heterogeneous. There will always be enough pores of sufficiently small size to retain coarse primary particles, but unless the texture is very heavy the truly colloidal clay will not tend to accumulate much within the profile.

EFFECT OF ELECTROLYTES ON PAN FORMATION

Sieve action by sand or silt skeletons affects mainly the large clay particles. If one were able to increase the size of the colloids, pan formation would likely

be enhanced. One of the most effective means to bring about aggregation in a colloidal system consists in the introduction of electrolytes. Since clay particles are negatively charged the dominating influence will be exerted by the positive ion (cation) of the electrolyte. Table 4 contains the flocculation values of a number of electrolytes expressed in terms of symmetry concentrations (S).[2] According to table 4 both the charge and the size of the cation are influential, though the former exceeds the latter in effectiveness.

TABLE 3

Flocculation of Putnam clay with chlorides

(Flocculation value expressed in terms of symmetry concentration)

FLOCCULATION CATION			FLOCCULATION VALUE NH₄-CLAY
Symbol	Valence	Size in Å.U.	
Na............................	1	0.98	7.0 S
Li............................	1	0.78	6.5 S
K.............................	1	1.33	5.0 S
NH₄..........................	1	1.45	4.5 S
Rb............................	1	1.49	.....
Cs............................	1	1.65	2.6 S
H.............................	1	....	0.87 S
Mg...........................	2	0.78	1.1 S
Ca...........................	2	1.06	1.1 S
Sr............................	2	1.27	1.1 S
Ba...........................	2	1.43	1.1 S
La............................	3	1.22	0.90 S
Th............................	4	1.10	0.75 S

TABLE 4

Salts used for pan formation with Na-clay

ELECTROLYTE	VALENCE OF CATION	FLOCCULATION VALUE	FLOCCULATION CONCENTRATION
NaCl........................	1	17.5 S	1 F
CaCl₂.......................	2	1.4 S	1 F
La(NO)₃.....................	3	1.0 S	1 F
ThCl₄.......................	4	0.85 S	1 F

The rôle of electrolytes in pan formation was studied for four different salts (table 4) which furnish cations of various charges. The electrolyte concentrations added to the clay systems (before their passage through sand) are expressed in terms of flocculation values, F, a procedure which facilitates graphical representation as well as interpretation of the results. The way

[2] The value of $S = 7$ for NaCl means that for a given set of conditions at least 7 times S milliequivalents of NaCl must be added to produce flocculation. The letter S represents the number of adsorbed (exchangeable) ions in the system under consideration.

in which increasing salt concentration changes the rate of flow is demonstrated in figure 3. To permit comparative analysis between the various electrolytes, figure 4 has been constructed, which forcefully brings out the pronounced effect of the charge of the cation. *The higher the charge the more rapid the reduction of the percolation velocity of the sol.* From the viewpoint of clay pan formation in nature, the difference between Ca and Na is particularly significant, since field observations indicate that many clay horizons are associated with lime concretions.

Special experiments on the flocculating power of CaCO₃ and CaSO₄ furnished conclusive evidence that both salts, in the presence of their solid phase,
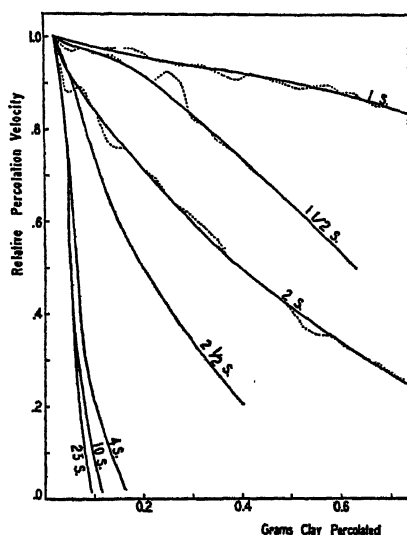
FIG. 3. EFFECT OF ADDITIONS OF CaCl₂ (SYMMETRY CONCENTRATIONS) ON THE RATE
OF PAN FORMATION

Abscissa: grams of clay as 0.3 per cent sol percolated through the sand column; ordinate: rate of pan formation which is inversely proportional to the relative percolation velocity of the clay sol.

coagulate the clay systems, no matter what the nature of the adsorbed cation (H, Na, K, NH₄, Ca, Mg).

Four samples of natural ground water from typical clay pan areas in Missouri and Illinois contained enough electrolyte to flocculate completely natural Putnam clay, even after three-fold dilution with distilled water.

Closer examination of figure 4 reveals the interesting fact that the different behavior of the various electrolytes persists up to concentrations many times the flocculation value. Apparently the cation does more than merely neutralize the charge of the particle; it also causes the formation of characteristic gross aggregates, which vary in size, density, and stability, and which impress their individuality on the kind and speed of pan formation.

Significance of hydration.—The floccules formed are relatively loose aggregates of strongly hydrated primary clay particles. During the passage through the sand the secondary aggregates may collide with quartz grains and temporarily break up into smaller units and thus retard accumulation. One would expect that dehydrating agencies, which remove the water shells and bring the primary particles into contact, would form more stable aggregates and thus accelerate pan formation. Experiments support this concept. Addition of
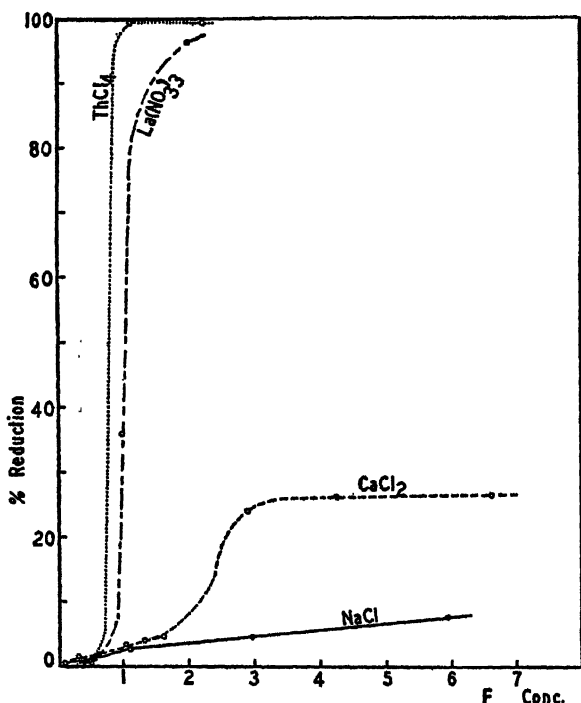
FIG. 4. INFLUENCE OF THE VALENCE OF THE CATION ON THE RATE OF PAN FORMATION

Ordinate: reduction of initial percolation velocity by 0.021 gm. of clay (in form of 0.3 per cent sol); abscissa: electrolyte concentration in terms of flocculation values.

alcohol to Na-clay in the presence of $CaCl_2$ and NaCl enhances the building up of impervious clay horizons in the sand column.

Rôle of protective humus.—Colloidal humus exerts protective action on suspensoids, i.e., it shifts the flocculation value of electrolytes to higher concentrations, as seen in figure 5. To flocculate 5 cc. of neutral Putnam clay sol for a given set of conditions, 0.1 m.e. of NaCl is necessary. Mixing the clay sol with neutral humus extract prior to the addition of electrolyte increases the stability many fold. The action of humus to protect the clay sol from precipitation manifests itself in the rate of pan formation, as indicated in figure 6. Natural colloidal humus from a sample of muck was added to Na-clay

at the rates of 0.017 gm. and 0.033 gm. per gram of clay. Twice the symmetry concentration of CaCl₂ was added, and the systems were percolated
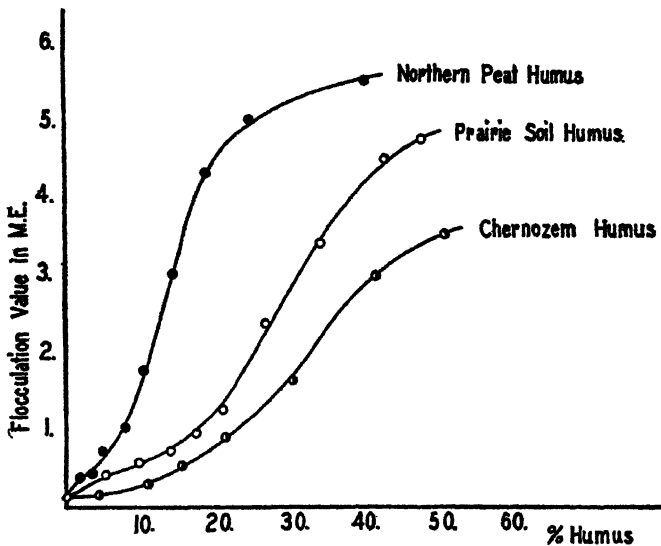
FIG. 5. PROTECTIVE ACTION OF COLLOIDAL HUMUS AGAINST FLOCCULATION OF NATURAL CLAY SOL BY NaCl
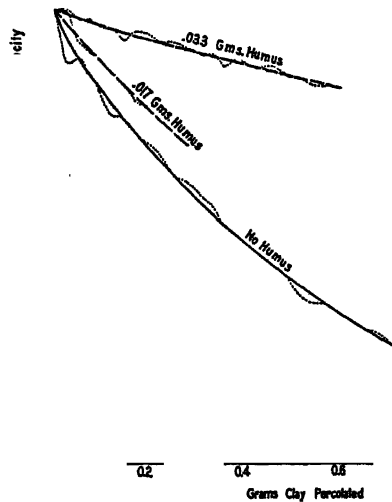
FIG. 6. REPRESSION OF PAN FORMATION BY COLLOIDAL HUMUS

through 20–40 mesh sand. The 0.017-gm. humus sample did not prevent flocculation, yet it retarded considerably the development of the pan. The

other sample fully protected the clay from coagulation and correspondingly prohibited the accumulation of the colloids (fig. 6).

Removal of coagulating salts.—If a pan is being formed in the presence of $CaCl_2$, for example, subsequent percolations of distilled water tend to reverse the process. With the removal of the excess of electrolyte the clay pan disperses and the rate of flow of the solution increases very rapidly; later it slows down and tends to approach the initial state.

Summarizing the rôle of electrolytes, we consider the following points to be most significant: The electrolytes in concentrations near or above the flocculation value cause the clay particles to unite to secondary aggregates, which are retained in the sand by mechanical sieve action. The process is reversible to a very large extent. Colloidal humus favors migration of clay particles.
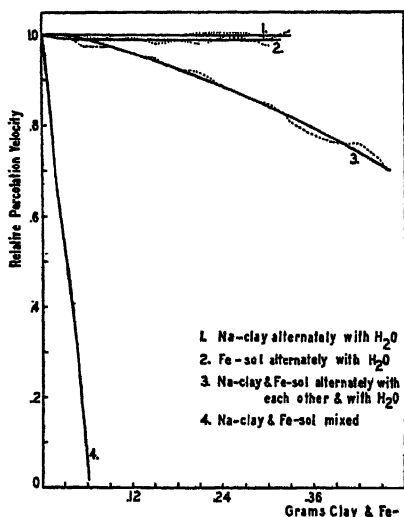
FIG. 7. FORMATION OF ATTRACTION PANS WITH THE AID OF POSITIVE IRON SOLS
$[Fe(OH)_3 \cdot x H_2O]$

Clay accumulations formed under the influence of coagulating electrolytes we shall designate as *electrolyte pans.*

THE ATTRACTION PHENOMENON IN PAN DEVELOPMENT

If a positive iron sol $[Fe(OH)_3 \cdot x H_2O]$ is passed through a column of spherical glass beads no change in the rate of flow can be detected (fig. 7). However, a thin coat of the Fe-colloid sticks to the beads (or sand grains) and—in contrast to clay particles—can not be washed off with water. Evidently the negative silicate spheres and the positive iron hydroxide particles attract each other and stick together permanently.

A similar attraction phenomenon occurs if positive iron sols and negative clay suspensions are mixed in suitable proportions. Mutual flocculation takes

place and pan formation results at once (curve 4, fig. 7). A fundamental difference exists between this "attraction pan" and the electrolyte pan in that the latter is largely reversible, whereas the former is not. Prolonged leaching with distilled water fails to disperse significantly the illuvial iron-clay horizon.

The two experiments just described can be combined to yield an interesting and unsuspected mode of pan formation. Ten cubic centimeters of 0.3 per cent dialyzed Fe-sol is permitted to pass the skeleton of negative glass beads. The iron hydroxide particles not held by the spheres are leached out with five successive washings of 10 cc. H_2O. Determination of the endoelectrosmotic flow reveals that the entire system has now become electropositively charged. Then 10 cc. of 0.3 per cent Na-clay is percolated through the same bed and the burette again rinsed with water to remove the free particles completely. Electrical measurements show that the entire system has turned negative again. The same process is repeated in the order: iron sol + water + clay sol + water + iron sol + ... etc. The electric charge of the system alternates regularly, always assuming the same sign as the sol that passed through last. Corresponding determinations of the rate of flow of water and sols indicate definitely that a pan is gradually being formed (curve 3, fig. 7). Obviously pan development is due to the building up of alternating layers of iron hydroxide and clay which restrict the width of the pores and reduce the rate of flow of the liquids. Sieve action may finally result.

As in the case of electrolytes, *colloidal humus* protects the sols also from mutual flocculation. If sufficient colloidal humus is added to a clay suspension, addition of Fe-sol may not produce any flocculation. On the other hand, precipitated iron-clay colloids in the form of coarse aggregates readily disperse upon addition of humus (with gentle shaking) to a finely divided, very permanent sol. It is of further significance that colloidal humus reduced the attraction between iron colloids and quartz grains.

The experiments on mutual flocculation lead to the conclusion that in spite of the absence of electrolytes pan formation can take place, even in coarse-textured systems, provided that positive colloids are present. The attraction pans are much more stable than the electrolyte pans.

APPLICATION OF EXPERIMENTAL RESULTS TO THE THEORY OF CLAY PAN
FORMATION

The concept of the ideal soil.—Workers engaged in experimental studies of soil formation are handicapped by the lack of soil types the origin of which is completely understood. The authors have found it convenient to think in terms of an *ideal soil profile*, which can be synthesized at will by the operation of but a limited number of soil-forming factors. The parent material of such a soil is supposed to have the following properties: It is made up of uniform spherical particles of fine sand size, and the chemical and mineralogical composition is that of the average values of the igneous rocks. To visualize pan formation in such a system, topography is assumed to be level and the

underdrainage good. Climate will be taken as of the arid-humid transition type on one hand, and of the strictly humid temperate type on the other.

Clay pan formation in the arid-humid transition zone (idealized conditions).— Rain water penetrates the parent material; hydrogen ions of water and CO_2 liberate the bases of the crystal lattices; the minerals break up; and clay particles are formed. The percolating rain water carries the easily soluble substances into the lower part of the profile where they accumulate (carbonate horizon) as a consequence of water absorption, evaporation, and reduced CO_2-pressure. Since rainfall is limited, no water will ever move out of the solum. As soon as the electrolyte concentration in the surface becomes sufficiently low, its clay particles will disperse and move downward with the water currents until they reach a zone where the salt accumulation is high enough to cause flocculation and retention of aggregates by sieve action. Additional clay migrating downward will also be coagulated, or, finding the pore size restricted, filtered out mechanically. In such a soil profile the chemical composition of the clay particles within the various horizons would be expected to be rather homogeneous. It is quite possible that the process just described explains the origin of clay pans in chernozems (1); in other words, they would be called *electrolyte pans.*

Clay pan formation under humid temperate conditions.—According to Norton and Smith (4) the most impervious clay pans in Illinois are found in regions of level topography associated with poor drainage (Cisne silt loam, Putnam silt loam). These clay horizons probably fall into the category of electrolyte pans. They differ from the chernozem phase in general in their more advanced weathering and more pronounced translocation of colloids, and in particular as a. result of the fact that flocculation is brought about by the electrolytes of the ground water.

In the absence of temporarily high water tables, clay pan development in humid regions is difficult to understand, unless the attraction phenomenon is taken into consideration. Under conditions of high precipitation the soluble electrolytes are constantly removed by the percolating rain water and the chances for the development of a permanent electrolyte pan are remote. However, the intensified weathering that produces large amounts of clay minerals (3) also favors the formation of positive iron and aluminum hydroxides. Throughout the profile they become attached to the negative quartz grains and, moreover, attract wandering clay colloids, all of which leads toward the development of an attraction pan. In the surface—on account of the protective organic matter—the downward migration of the colloids is enhanced and the differentiation of the soil profile into A- and B-horizons becomes accentuated. In well-drained soils one would not expect very pronounced clay zones, but rather a distribution of the particles over a greater depth. Furthermore, the clay colloids (in contrast to the chernozem pan), would likely vary considerably in their chemical composition, particularly in the silica-sesquioxide ratio.

The modes of pan formation outlined apply to ideal soils, but nature never provides such simple systems. The heavy clay pans observed in temperate regions have undoubtedly developed by combinations and modifications of the "electrolyte" and "attraction" principles.

SUMMARY

For the study of clay pan formation a method has been developed which permits quantitative measurements of clay accumulations in sand columns.

Putnam clay, if sufficiently dispersed, will not form a heavy pan in beds of quartz sand or silt by mere sieve action.

Electrolytes are responsible for the development of "electrolyte pans" as a result of flocculation and retention of coarse clay aggregates. The higher the valency of the electrolyte cation the more rapid is the pan formation. Dehydration enhances clay accumulation, whereas colloidal humus favors translocation.

Positive iron hydroxide sols give rise to "attraction pans" based on adherence of iron hydroxide particles to the negative quartz grains and on mutual coagulation with negative clay colloids.

Principles of clay pan development in ideal soils are discussed.

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SOIL PROFILE STUDIES: VII. THE GLEI PROCESS

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In Russia the popular understanding of glei, according to Vuisotzkii (5), is "a more or less compact, sticky loam or clay parent material, which is not, however, as sticky as the usual loam or clay, frequently with a more or less clearly pronounced light greenish blue tinge."

Glei formation is found in marshes and under conditions of a high water table. The glei horizon is generally water-logged, except when the water table recedes. In this horizon, therefore, anaerobic conditions prevail, which favor reducing reactions and minimize leaching effects. Besides, the ground waters enrich the glei horizon with bases, if such are in solution, and impart to it an alkaline reaction. In the reduction process the ferric iron gives rise to the ferrous iron, and in the case of the hydroxide the weak base changes into the stronger ferrous iron base. Partly because of this fact, therefore, one should expect a higher pH in the glei horizon. Data on the reaction in the glei horizon bear this out. With the receding of the level of the water table, the ferrous iron is oxidized and the pH must naturally drop. This perhaps is the reason why some glei horizons show a lower pH than others.

The simultaneous downward and upward movements of the substances in solution bring about a unique condition with respect to the iron therein contained. The anaerobic state is conducive to reducing reactions and the insoluble iron compounds become soluble and move downward. At the same time the rise of substances in solution by the capillary forces brings back some iron to a point where it might come in contact with the air and precipitate. We thus find an ocherous layer on top of the glei horizon. This is probably the chief factor in the process of bog iron formation. While the upper layer of the glei horizon becomes enriched with iron, the lower layer becomes impoverished of iron. It is in this layer of the glei horizon that we find the mottling effects with the characteristic gray-bluish-green tinge of the material.

In many respects the process of ochre formation is similar to that of the rising of salts in alkali soil formation. This has been pointed out by Vuisotzkii (5), who was probably the first to study glei formation.

Within the podzol zone, especially in its northern belt adjoining the forest tundra, glei formation is not uncommon. This does not exclude its existence in other soil zones, since it is not a climatogenic formation, but a hydrogenic. Indeed Vuisotzkii (5) points out the presence of glei formation in the southeastern Precaspian steppe.

Glei formation has been studied primarily in the podzol zone and has therefore been associated with this zone. Whenever glei formation is found on a podzol soil, it is the C or B horizons that undergo the respective changes. The A_2 horizon remains intact, i.e., the rising waters do not reach this horizon.

Frosterus (1), in his work on the podzols with clay as parent material and a high water table, discusses the question of glei.

An extensive study of soils with a glei horizon in the podzol zone has been made under the direction of the late Glinka by Zavalishin (7).

TABLE 1

Reaction of soils with a glei horizon

(After Zavalishin)

SOIL	HORIZON	DEPTH	REACTION
		cm.	pH
Clay marsh...........................	A_0	0– 8	6.2
	A_1	8–18	6.5
	A_2	18–29	6.5
	G	29–42	5.9
	G	42–61	6.5
	G	61–75	7.4
Clay marsh...........................	A_1	0–10	5.8
	A_2	10–25	5.8
	G	25–60	7.3
Clay marsh...........................	A_0	0– 8	5.7
	A_1	8–22	6.2
	G	31–61	· 6.5
Podzolic-glei...........................	A_1	0–20	5.8
	A_2–G	20–28	6.4
	G	28–40	6.5
Podzolic with a glei horizon..................	A_1	3–14	5.4
	A_2	14–36	5.8
	B–G	36–56	6.8
	G	56–65	7.0

In table 1, adapted from Zavalishin (7), the reaction of a number of soils with a glei horizon is given. In table 2 the soluble humus and the total humus are recorded.

It will be noted that with depth the soils become less acid and the glei horizon reaches the neutrality point. There is not much organic matter in the glei horizon, but the quantity of soluble humus increases with depth and is higher in the G horizon than in the overlying A horizon, which has a higher total of humus.

An interesting attribute of the glei horizon is the base saturation of its base-

TABLE 2

Water-soluble humus in soils with a glei horizon

(After Zavalishin)

SOIL	HORIZON	TOTAL HUMUS	KMnO₄ USED FOR WATER-SOLUBLE HUMUS	SOLUBLE HUMUS
		per cent	*cc.*	*per cent*
Peat podzol; podzolic glei..............	Humus ortstein (bog-iron)	2.25	2.55	0.00033
Peat podzol; podzolic glei..............	G (upper portion)	1.35	7.11	0.00093
Glei marsh...........................	G	0.23	5.10	0.00066
Podzol, with ortsand..................	Humus ortsand	4.19	4.40	0.00057
Peat podzol...........................	Lower portion of humus ortsand	2.16	10.20	0.00132
Peat podzol...........................	G₁	0.51	8.15	0.00105
Peat podzol...........................	G₂	0.41	8.35	0.00108
Peat marsh...........................	G	0.34	7.85	0.00102

TABLE 3

Exchangeable bases in base exchange complex of glei soils

(After Zavalishin)

SOIL	HORIZON	ABSORBED CATIONS			Mg IN TERMS OF Ca	H IN TERMS OF Ca	TOTAL CATIONS IN TERMS OF Ca
		Ca	Mg	H			
		Per cent of dry soil					
Clayey marsh on a heavy loam...................	A₁	2.15	0.16	None	0.27	.....	2.42
	G₁	0.36	0.11	None	0.16	.....	0.62
	G₂	0.30	0.12	None	0.19	.....	0.49
Podzolic glei sandy loam.....	A₁	0.12	0.02	0.0006	0.03	0.012	0.16
	A₂	0.05	0.005	None	0.01	.....	0.06
	G	0.07	0.007	None	0.01	.....	0.08
Podzolic soil with a close glei horizon.................	A₁	0.17	0.04	0.0008	0.06	0.016	0.25
	A₂	0.06	0.02	None	0.03	.....	0.09
	B	0.14	0.06	None	0.10	.....	0.24
	G	0.18	0.10	None	0.17	.....	0.35
Weakly podzolized with a close G horizon on a heavy loam.................	A₁	0.76	0.23	......	0.38	.....	1.14
	A₂	0.23	0.04	......	0.06	.....	0.30
	B–C	0.24	0.12	......	0.20	.....	0.44
	G	0.24	0.15	......	0.25	.....	0.49
Weakly podzolic glei loam....	A₁	0.38	0.08	......	0.13	.....	0.51
	A₂–G	0.13	0.05	......	0.08	.....	0.21
	G	0.18	0.08	......	0.13	.....	0.31

exchange complex.   As indicated in table 3, adapted from Zavalishin, the glei horizon differs but little from the parent material except that it contains slightly less Ca.   Its Mg is less mobile than the Ca and in absolute terms the glei contains more Mg than Ca, the reverse of which is true for normal soils.   In regions where the ground waters are rich in K and Na, the glei horizon is more sticky and is in a poorer physical condition than an ordinary glei.   A case of this nature has been described by Wityn (6), who found the acid and normal

TABLE 4

Al_2O_3 and SiO_2 extracted from glei podzols with 0.05 N HCl and 5 per cent KOH
(After Zavalishin)

SOIL	HORIZON	ON REPLACING THE BASES BY EXTRACTING WITH 0.05 N HCl		SOLUBLE IN 5 PER CENT KOH EXTRACT		MOLAR RATIO OF Al_2O_3: SiO_2 FROM KOH EXTRACT
		Al_2O_3	SiO_2	Al_2O_3	SiO_3	
		per cent	*per cent*	*per cent*	*per cent*	
Clayey marsh on a heavy loam....	A_1	1.10	Traces			
	G_1	0.40	0.12	0.36	0.44	1:2.09
	G_1	0.46	0.13			
Clayey marsh loam.............	A_1	0.30	0.028			
	G	0.18	0.025	0.38	0.63	1:2.83
Podzolic glei loam..............	A_1	0.30	0.012			
	A_2–G	0.16	Traces			
	G	0.16	0.020			
Podzolic glei sandy loam.........	A_1	0.15	Traces			
	A_2	0.08	Traces			
	G	0.10	0.01			
Podzolic loam with a close G horizon....................	A_1	0.18	Traces			
	A_2	0.056	Traces			
	B	0.16	0.04			
	G	0.40	0.14			
Weakly podzolized with a close G horizon on a heavy loam.......	A_1	0.54	....			
	A_3	0.16	Traces			
	B–G	0.30	0.08			
	G	0.38	0.24	0.42	0.81	1:3.1

carbonates in the glei horizon.   It is of interest that the horizon overlying the glei horizon is relatively high in absorbed Ca and Mg.   This condition is probably due to the capillary rise as the water table rises.

Another interesting attribute of the glei soil is its SiO_2 and Al_2O_3 content soluble in 0.05 N HCl (Gedroiz acid) and in 5 per cent KOH solution (Gedroiz method).

From table 4, adopted from Zavalishin, it is clear that the SiO_2 content

extracted with the 0.05 N HCl increases with depth. Practically none, however, is found in the filtrate from the podzolized A_2 horizon, notwithstanding the fact that its total SiO_2 content is highest, as compared with the other horizons. The same is almost true for the A_1 horizon. The highest quantity of SiO_2 goes into solution from the glei horizon after the Ca has been replaced. To appreciate more fully the significance of the data, Zavalishin presents figures on the SiO_2 extracted from other soils by the same method:

The B horizon of a sandy podzol from the Leningrad district gave 0.06 per cent of SiO_2; the B horizon of a loam from the same district, 0.03 per cent; an ortsand of a sandy podzol from the Valdai county, 0.01 per cent; and ortstein from Pavlovsk, 0.08 per cent; the B horizon of a red soil from the Bengaza steppe desert, 0.32 per cent; an ortstein of a podzol sandy loam in the Murman region on the river Tuloma, 0.18 per cent.

Leaving out of our discussion the desert soil, for the reason that the soil forming processes are entirely different from those in the podzol zone, we can readily see that, as a rule, the SiO_2 soluble in HCl from the true podzol or podzolic horizons lags far behind that of the glei horizon. Apparently there is some decomposition of the base-exchange complex in the glei horizon. Zavalishin adds that "probably some amorphous SiO_2 is carried to the glei horizon from the upper horizon and from the ground waters."

As to the HCl-soluble alumina, Zavalishin points out that

... it is higher than in A_2 and in most cases lower than in A_1. In the B horizon the soluble Al is even higher than in the A_1 horizon.

Turning our attention to the results of the alkali, 5 per cent KOH, extracts [Gedroiz (2) method], it is to be noted that in the soils investigated the molar ratio of $Al_2O_3:SiO_2$ fluctuates between 1:2.09 and 1:3.8. Thus there is a slight excess of SiO_2. This indicates the probability of splitting off SiO_2 by the action of the ground waters.

One other important feature in the gleiing process is the presence of ferrous iron in the glei horizon. Zavalishin investigated a series of soils and presented his findings in a series of tables. His summary of these follows:

1. No water-soluble ferrous compounds have been found in the soils investigated. 2. The lower hydroxides make up the bulk of FeO found (as determined on H_2SO_4 extract). Some ferrous compounds of phosphoric acid are encountered. Small quantities of ferrous compounds of sulfur are found. 3. The highest quantity of FeO is found in the G horizon of the clayey marsh loam soil (1.4 per cent), the smallest in the transition horizons A_2-G and B-G of the podzolic soils with a close glei horizon. 4. The quantity of ferrous iron does not go beyond the limit of 20 per cent of the total iron. 5. The solubility of the FeO compounds varies in the different horizons which are subjected to the gleiing process; it is highest in the upper parts of the glei horizon, in A_2-G and B-G. 6. Not all the forms of FeO compounds in the soil are easily oxidized in the air.

In table 5 Zavalishin presents data on the total constituents of the horizons of a number of glei profiles. He makes the following remarks:

Although the data show no sharp fluctuations in the composition of the different horizons, a number of points are worthy of notice. Of all the constituents the Al_2O_3 seems to be the

most mobile. It is clear that A_2 becomes impoverished and B enriched with Al_2O_3, while the least mobility takes place in the glei horizon. The iron does leach slightly from the G horizon, but in these samples it is weakly expressed. In the podzol sandy types the glei horizon occupies a position intermediate between the true podzol horizon and the parent material, suffering, however, a definite loss of iron. It appears that iron goes into solution only either at the outset of the gleiing process or periodically when the horizon becomes saturated with water enriched with organic substances and primarily with carbonic acid. This phenomenon finds its corroboration in the separation of iron formations in the form of bog iron ore at the lower points of the clayey and peat-glei bogs. In these places the ground waters as they come to the surface become enriched with humus and their products of decomposition.

TABLE 5

Total analyses of several glei podzol soils

(After Zavalishin)

SOIL	HORI-ZON	ON BASIS OF DRY SOIL			ON BASIS OF MINERAL PORTION OF SOIL				
		Hygro-scopic water	Chemi-cally com-bined water	Humus	SiO₂	Fe₂O₃	Al₂O₃	CaO	MgO
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Podzolic loam with a close glei horizon..............	A_1	4.93	12.69		73.28	3.82	16.85	2.28	1.84
	A_2	1.25	1.19	1.28	77.48	3.40	13.86	1.84	1.52
	B–G	1.29	2.53	0.40	72.18	2.50	18.44	2.62	1.65
	G	1.75	1.47	0.30	75.10	3.15	15.42	2.84	1.50
Podzolic loam with a close glei horizon..............	A_1	3.56	10.64		74.24	3.05	14.85	2.76	1.94
	A_2	2.02	4.50		77.35	3.08	13.40	1.88	1.46
	B	2.65	2.20	0.42	74.25	5.25	14.27	2.00	1.50
	G	2.30	1.88	0.20	74.50	3.18	16.30	1.92	1.64
Podzolic sandy soil with a close glei horizon.........	A_2	0.10	....	....	93.42	0.48	6.08	0.30	0.09
	G	0.25	....	....	92.55	0.62	7.25	0.50	0.14
	C	0.30	....	....	89.92	1.20	7.85	0.80	0.25
Weakly podzolic sandy soil with a glei horizon........	G	0.20	....	....	91.25	0.70	6.90	0.50	0.13
	C	0.30	....	....	90.01	1.00	7.15	0.60	0.11

The bases leach but slightly from the G horizon, a great deal less than from A_2. In general there is not much loss of the alkaline earths, the Mg being particularly stable.

Thus the total analyses seem to indicate a certain similarity between the glei and podzolized horizons. The decrease of hygroscopic and chemically combined water in the glei horizon points in the same direction. The similarity, however, is slight and chemically the glei hori- zon resembles more the parent material than the podzol. Fluctuations in the total analyses of the glei horizon are, of course, probable. These depend in a large measure on the movement of the moisture which brings about the gleiing effects, i.e., the rapidity with which the solution which acts on the soil changes. It is therefore clear that sandy loam glei horizons, because of the aforementioned, will undergo more change than clay horizons. The process of removing substances in the gleiing reactions is a secondary phenomenon. The primary phenomenon— the change from the higher to the lower states of oxidation—finds a higher expression in clays

than in sands. It is well to remember that other soil horizons, even the A_2, might be subject to the gleiing process with the change in the level of the water table. A chemical analysis of such a horizon may lead to wrong interpretations.

OXYGEN AS A FACTOR IN THE PROCESS OF GLEI FORMATION

Tamm (4) investigated the oxygen tension of the ground waters in a number of soils which are affected by a high water table. His highly interesting results have a bearing on the problem of glei formation. In table 6, compiled from Tamm's data, a number of points are brought out.

TABLE 6

Oxygen content of ground waters in soils related to the marsh or bog type of soil formation

(Compiled from Tamm's data)

SOURCE OF SAMPLE	DEPTH	OXYGEN PER LITER OF WATER	SOURCE OF SAMPLE	DEPTH	OXYGEN PER LITER OF WATER
	cm.	*cc.*		*cm.*	*cc.*
Open body of water........	...	8.05		120	0.00
	...	8.35		120	0.08
			Edges or fringes of marshes with a thin layer of peat..	55	0.12
Moraine soil in neighborhood of open body of water.................	245	3.21		55	0.12
	245	3.72		100	0.34
	245	3.80		100	0.35
	...	3.96			
	30	1.61		80	4.39
	30	0.28	An island of dry soil in a marsh................	120	2.15
Under soils with a deep humus layer with spots of peat and a moss cover....	105	2.53		185	0.42
	105	2.78		225	0.00
	30	0.00			
	30	0.00	Belt of soil bordering the marsh, with a 15–25 cm. peat layer.............	30	0.32
	85	3.04		40	0.05
				60	0.54
	63	3.99		100	1.30
	95	4.68		130	2.20
Areas of peat bordering the extensive marshes.......	95	5.82			
	66	0.00			
	85	1.98			
	70	2.21			
	95	2.70			

A comparison of the oxygen content in the ground waters of moraine soils with that of open bodies of water, springs, and streams, shows that the former contain less dissolved oxygen. However, if we compare the dissolved oxygen content of moraine soils with that of soils containing a deep humus layer with spots of peat and a moss cover, we find that the moraine soils are fairly rich in dissolved oxygen. In the peat areas bordering the extensive marshes the ground waters are rich in oxygen, except at one point, at a depth of 66 cm. On the other hand, the ground waters of the fringes of the marsh, having a thin

layer of peat, are free from, or very poor in, oxygen. Similarly the ground waters of the belt of soils bordering the marsh which, however, have a layer of peat 15 to 25 cm. thick, show a very low oxygen content. According to Tamm, the apparent discrepancies are due to the movement of the ground waters. Whenever there is a free movement of the ground waters from the dry areas through the marsh, there is oxygen even under the peat. In the case of stagnant waters the oxygen is utilized as it penetrates the peat, and the ground waters show a low oxygen content. Tamm points out that rain water percolating through peat loses its oxygen, whereas through a normal humus horizon it is carried downward. Zavalishin (7) cites areas of the latter type with poor stands of pine under which the soil has undergone the effects of gleiing.

Tamm also shows that in cases where the ground waters from a marsh, because of topographic and geologic conditions, flow through coarse-textured strata, ortstein formations appear which ultimately clog the underground channel. Apparently the reduced iron compounds in the marsh waters become oxidized, precipitate, and accumulate as "allochtonic" ortstein (according to Tamm's nomenclature).

Zavalishin (7) infers from Tamm's results and his own observations that the

... oxygen determinations of the ground waters may explain the frequent failures of forest stands in certain areas, the formation of ortstein in conjunction with such areas, and the formation of the glei horizons. According to Tamm the water which percolates through a peat horizon, 40 to 50 cm. deep, is free of oxygen and when such a water system meets an underground stream saturated with oxygen we have two water courses unrelated hydrostatically. This might explain why the more level bluish glei horizons free from iron concretions are encountered under the clayey or peat-clayey horizons A_0 and A_1. They are the least penetrable by the oxygen. At any rate no ortstein formations are found under clayey or peat horizons.

Zavalishin sums up the discussion on glei as follows:

The glei horizon appears in the form of a sandy or clay material of a light-gray or gray color with a bluish, blue, or sky-blue tinge. The color is not uniform; it depends on the intensity of gleiing and on the mechanical composition of the material. Usually the gray-blue background is mottled with large red spots and veins. Usually these spots are associated with the cracks and root paths and they are more frequent in the clay varieties. The spots around the roots may be of two kinds: if the material is not strongly gleied and there is decomposed organic substance in the root path, then there develops a light-gray-bluish glei formation with a reddish band on the outside. If, on the other hand, the gleiing has proceeded very far and the root path is nothing more than a tube through which air passes, then a red ring forms around it on a light-gray-bluish background. When the gleiing is very strong, the parent material is of a homogeneous gray-blue coloration without any spots or veins. Glei horizons, especially sandy, at times resemble podzols; the bluish tinge and the red spots identify it, however, as glei.

Usually the glei horizons are without structure, more or less compact, sticky, smeary, and appear to be more clayey than the adjoining parent material. A suspension of glei formation does not settle out and it is highly dispersed.

The humus content is higher in the upper layer of the glei horizon than in the lower. The solubility of the humus increases with an increase in gleiing. With that, the reducing property of the glei increases and this in turn influences the reducing reactions with respect to the iron compounds.

A GLEI-LIKE PODZOL IN NEW JERSEY

A study has been made on the effect of a high water table in a podzol sandy soil of the Collington series in New Jersey. The profile sampled is in the Freehold area in the soil survey of New Jersey, between the parallels 40°18′ north latitude and between the meridians 74°08′ and 74°10′ longitude. The profile cut was made in a wooded section just about where the road running north and south in the particular sector crosses the Hockhockson Brook.

Topographic and climatic features.—The topography of the section is level, with minor microrelief features. Geologically it belongs to the Coastal Plain Province, which consists of formations of unconsolidated, almost horizontal beds of gravel, sand, sandy clay, and marls, with a slight dip to the coast. The formations are chiefly Tertiary, the upper Miocene. The climate is characterized by the relatively narrow daily and monthly ranges of temperature. The rainfall is rather heavy, averaging about 50 inches annually, with a minimum of about 40 and a maximum of over 70 inches. The mean annual temperature is about 53°F., with an absolute minimum of −11°F., and an absolute maximum a little over 100°F. During the winter the ground freezes occasionally a few inches deep, but these frosts do not last very long and are alternated by mild weather, which quickly thaws out the frozen ground. The humidity is relatively high except during some periods in the fall. An important feature of the rain-fall element of the climate is its relatively even distribution.

The forest vegetation consists of a stand of second growth hardwoods, 20 to 50 years old: red maple, black and white oak, sweet gum, gray birch, and ironwood. Of the more prominent herbaceous vegetation noted are: smilax, carex, cinquefoil, and star flower.

Morphological characteristics.—The morphological features of the profile under consideration are as follows:

A_0: 0 to 2 cm. deep; forest débris of dead leaves and twigs, some slightly decomposed and some still fresh.

A_1: 10 cm. deep; dark gray with a sprinkling of white silica; structureless; sandy texture; charcoal found in upper portion.

A_2: 25 cm. deep; light brown; sandy texture but heavier at the bottom; structure tending towards lamination; poor in organic matter.

B: 15 cm. deep; reddish brown (coffee color); slightly heavier than A_2; iron incrustations found; more distinctly expressed structure in upper portion.

C: A typical glei formation; color grades into a bluish gray; dark iron concretions are scattered through the horizon; tongues of iron formations penetrate into this horizon. Greensand marl is found below a depth of 65 cm., with the water table, at the time of examination, at that point.

Chemical characteristics.—In table 7 data are presented on the chemical composition of the soil.

The outstanding feature of the data is the high acidity of the soil. Both the pH and the unsaturation clearly show that with depth the acidity increases. This is not in accord with the findings of Zavalishin and the other Russian investigators who claim that the G horizon, as a rule, is less acid than the

overlying horizon. Apparently the composition of the ground waters is a factor in determining the reaction of the glei horizon. In this particular case the ground waters from the adjoining areas have proved to be acid, pH 5.2 and even lower.

If it were not for the absence of a morphologically apparent A_2 horizon, this profile could be interpreted chemically as being a genuine podzol. The high SiO_2 content in A_2 and the accumulation of R_2O_3 constituents in B testify to that. There are, however, a few departures from the true chemical features of a podzol. Thus, for instance, the Ca content is not higher in the B than in the A_2 horizon, the *usual* case in podzols. Neither is the pH of the B horizon typical of a true podzol, in which it is usually higher than in the A_2 horizon. And apparently all of this is affected by the gleiing process, which incidentally is also evident from the morphological description of the B horizon; instead of being 40 to 50 cm. thick, as it is in the adjacent normal podzols, the Lakewood soil series, which was described in an earlier publication (3), is only 15 cm. thick. Apparently the gleiing process has acted on the B horizon, and the

TABLE 7

Chemical composition of a glei podzol sandy soil in New Jersey

	pH		SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	K_2O	N	BASE EX-CHANGE CAPACITY	UN-SATURATION (H-IONS)
HORIZON	Water extract	Neutral salt solution extract									
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*m.e.*	*m.e.*
A_1............	5.2	4.2	84.79	1.38	2.1	0.264	0.190	0.360	0.173	14.38	13.17
A_2..........	5.4	4.6	90.69	1.73	3.5	0.216	0.272	0.485	0.0485	6.36	3.67
B...........	5.1	4.4	83.82	2.55	6.23	0.189	0.282	0.420	0.0745	9.56	6.55
G...........	4.9	4.6	84.23	3.08	5.15	0.185	0.363	0.545	0.0855	9.92	8.45

slight variations in the chemical composition of the B and G horizons might be ascribed to that. It is of interest to note that the Mg accumulated in the G horizon, a typical attribute of the gleiing process. The high K content is probably due to the glauconite in the greensand marl, the parent material of this profile.

The Fe_2O_3 content is high in the B horizon as well as in the G horizon. One might look upon this accumulation of iron as a result of the combined action of the process of illuviation and gleiing. No ferrous iron could be demonstrated in the B horizon, but the G horizon gave a distinct reaction for ferrous iron immediately after the profile was sampled. Upon exposure to the air and drying the ferrous iron was oxidized.

The high humus content in the B and G horizons, as evidenced by the high N content, indicates a movement of this constituent from the A horizon. It undoubtedly moves in colloidal state by mutual protection with he iront. With the high iron content in these horizons the ortstein formation noted must be classified as an iron-humus ortstein.

The similarity in base-exchange, or rather cation-exchange, capacity in the B and G horizons is proof of the gleiing process extending its influence also on the B horizon, even though this is not apparent morphologically. The increased base-exchange capacity in the B horizon, in comparison with the A_2, is one of the properties of a podzol.

General statement.—The profile under consideration is located at the fringe of a swampy area, and there are indications that the water coming in from a higher level contains appreciable quantities of dissolved oxygen. The ortstein veins, the orterde concretions, and the tongue-like iron formation projections in the G horizon are ample evidence of the high oxygen content in the ground waters which tends to obliterate some of the gleiing effects.

We may thus look upon this soil as a glei-like podzol, a sort of transition to the peat podzol which adjoins this particular area. After all, the process of gleiing and the marsh type of soil formation, of which the peat podzol is one of the subtypes, are closely related.

At the edge of the podzol zone, in the northern hemisphere, where the forest vegetation begins to dwindle, more and more of the glei process is apparent, more and more is the marsh type the outstanding or prevailing type of soil formation. *Thus the glei podzol in the northern countries testifies to the termination of the podzol process of soil formation and the inauguration of the northern marsh or tundra type of soil formation.*

SUMMARY

A review and discussion of the meager work on gleiing, with special reference to the important investigations of Zavalishin, have been given.

The effect of oxygen in the ground waters on the process of gleiing and incidentally also on the state of the iron compounds has been pointed out in the light of Tamm's work.

Analyses of a glei podzol in New Jersey have been presented and interpreted.

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BOOK REVIEWS

Kurs Pochvovedeniya (A Course in Pedology). By I. V. TYURIN. State Publishing, Moscow-Leningrad, 1933. Pp. 1–312.

In harmony with the general trend of publications in the U. S. S. R., the text-book of Tyurin treats the subject of pedology from the social-economic, or the so-called "economic determinism," standpoint. Of the rôle of soil in human relations, Tyurin states: "Because of this property [fertility] the soil, at a certain state of development of human society, becomes a means of production and an object to which labor is applied for the purpose of exploiting and increasing its fertility." The author does not maintain this viewpoint much beyond the introduction, although he returns to it here and there throughout the book; otherwise he develops the orthodox views of Dokuchaev. The ten chapters are as follows: I. The origin and composition of the mineral portion of the soil; II. Formation of the organic fraction of the soil; III. The process of the mutual activities of soil formation; IV. The soil as a medium for plant growth; V. Soil classification; VI. The soils of the forest regions of the U. S. S. R.; VII. The soils of the forests steppe and chernozem-steppe regions; VIII. The soil of the forestless regions of the U. S. S. R.; IX. The method of studying soils in nature; X. The physical and chemical methods in investigating soils.

This book is designed for students in the schools of forestry, and consequently very little consideration is given to soils outside the forest zone. Thus the gray, brown, and chestnut-brown soils are hardly mentioned; neither are the saline soils taken up in any detail. In six pages the author covers the soils of the dry and desert steppe and of the tundra. The subject of the forest soils, however, is treated in a clear, concise, yet thorough manner, with special attention to the processes involved in soil formation and to the reactions in connection with the base-exchange complex. The pedologist and, even more so, the forester interested in his substratum, the soil, will find this new contribution of great value.

J. S. JOFFE.

Kurs Sel'skokhozyaistvennogo Pochvovedeniya (A Course in Agricultural Pedology). By A. N. SOKOLOVSKII. State Publishing, Moscow-Leningrad, 1934. Pp. 1–271.

This book is a departure from the general books on pedology, inasmuch as it treats of the fertility elements of the soil; it is not a pedologic, morphologic, or geographic treatise.

The book is divided into eight parts: I. The problems of the science of the

soil and its rôle in socialistic agriculture; II. The origin of soils; III. Soil col-
loids and their dynamics; IV. The absorption properties of soils; V. The physi-
cal properties of the soil; VI. The chemical and biological processes in soils;
VII.   The origin of the soil types of the U. S. S. R. and their characteristics;
VII. Soil fertility.

Throughout the book the author attempts to harmonize the elements of the
pure sciences with the philosophical doctrines of the social-economic theories
of Marx, Engels, and Lenin, and it is to these theories only that the author
cites references.   There are no references to soils literature; neither will one
find illustrations, maps, or charts pertaining to soils.

The book is written in a very fine and clear style suitable for students who
are to be initiated into the science of soils.   The subject is treated from the
standpoint of the colloidal reactions in the soil with special reference to the
circulation of the calcium ion.   In this respect the book is more than a text
for beginners: the advanced student will find a comprehensive discussion of
the physicochemical reactions of soil colloids and of the perplexing problems
of soil fertility.

As an addition to the purely pedological treatises of Glinka, Zakharov,
Kravkov, and others, this book is to be highly recommended.

J. S. JOFFE.

THE FEEDING POWER OF PLANTS FOR THE POTASSIUM IN FELDSPAR, EXCHANGEABLE FORM, AND DILUTE SOLUTION[1]

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It is probably true, with a few exceptions, that 95 per cent or more of the potassium in soils exists in the form of feldspars and micas and is difficulty available. The readily available portion, consisting usually of not more than a few hundred pounds per acre in the plowed layer, exists in many soils almost entirely as exchangeable potassium.

Feldspars as a source of potassium for plants have been a subject of many investigations; for a review of the literature, the reader is referred to the articles by De Turk (3) and Haley (4). Because of variations in the amounts applied, differences in fineness of grinding, species of plants grown, and other experimental factors, the results reported by various investigators do not agree in all cases as regards the availability of feldspathic potassium.

The majority of investigators agree that exchangeable potassium is readily available. The literature regarding this matter is thoroughly reviewed in the recent papers by Hoagland and Martin (5) and by Magistad (10). The questions regarding the availability of potassium in exchange materials of varying degrees of saturation, however, have not been fully clarified.

The present investigation was undertaken to gain further information concerning the differences in the feeding power of various plants for the potassium in feldspars, in dilute solution, and in exchange materials of varying degrees of saturation.

PLANT FEEDING ON FELSPATHIC POTASSIUM

Methods

A study was made of the feeding power of 12 different crop plants for the potassium in feldspar. Two-gallon glazed earthenware jars, provided with a drainage hole, and filled with 10 kgm. of quartz sand, were used as culture vessels. The sand contained a trace of soluble potassium which was not washed out.

The availability of an application equivalent to 2 tons to the acre of 180-mesh microcline feldspar, thoroughly mixed with the sand, was compared to that of a 100-pound per acre application of potassium as KCl. The KCl was dissolved and added with the nutrient solution. Salts free of potassium were used for supplying the other nutrients. Phosphorus in an amount equal to 0.007 per cent of the weight of the sand was supplied by mixing chemically equivalent quantities of $Ca_3(PO_4)_2$ and $FePO_4$ with the sand. Two solutions, made up separately so as to prevent precipitation of calcium as the sulfate, were prepared to supply the other nutrients, namely:

(A) 118 gm. of $Ca(NO_3)_2 \cdot 4H_2O$
      Sufficient H_2O to give 1,000 cc. after solution
(B) 61.6 gm. $MgSO_4 \cdot 7H_2O$
      0.15 gm. $MnCl_2 \cdot 4H_2O$
      0.05 gm. NaI
      Sufficient H_2O to give 1,000 cc. after solution

To each jar were applied 20 cc. each of nutrient solutions A and B, after these portions had been added to, and mixed with, about 500 cc. of water. More was added when needed.

Seven to ten days after the seeds were sown, the plants were thinned to the following number per jar: Corn, 3; alfalfa, alsike clover, red clover, and sweet clover, 20; and the others, 10. Care was taken to leave uniformly vigorous and well-distributed plants in each jar. After 45 to 60 days' growth, all crops except alfalfa, alsike clover, red clover, and sweet clover were harvested. The latter were grown 120 to 140 days. The plant tissue was dried at 75° to 80°C. and weighed. Buckwheat, alfalfa, and sweet clover were grown during both the 1933 and 1934 seasons; the other crops were grown only during 1933. The results are given in table 1, and figure 1 of plate 1 is illustrative of the results obtained with sweet clover.

Duplicate samples were composited, ground, and analyzed for potassium in the following manner: A 2-gm. sample of tissue was placed in a porcelain crucible, moistened with 5 cc. of an alcoholic 40 per cent $Mg(NO_3)_2 \cdot 6H_2O$ solution, the alcohol burned off, and the charred tissue ashed over a low flame. The bulk of the ash was taken up with water, transferred to a 250-cc. volumetric flask, the crucible rinsed with 2 cc. of concentrated acetic acid, and the solution made up to volume. Potassium was finally determined by the cobaltinitrite procedure as outlined by Volk and Truog (16). The results are given in table 2.

Results

In calculating the comparative yields in table 1, the yields obtained with soluble potassium are taken as a standard and expressed by 100, and the other yields are expressed as percentages of the standard. The results show that plants differ as regards their feeding ability for feldspathic potassium. It was found that corn, rape, buckwheat, peas, sorghum, sudan grass, soybeans,

TABLE 1

Yields, in grams of oven-dry material, of 12 different crops grown in quartz sand cultures supplied with readily soluble and feldspathic potassium

TREATMENT	YIELDS OF DRY TISSUE IN GRAMS			COMPARATIVE YIELDS
	A	B	Average	

1933 results

Corn

Soluble potassium	21.5	...	21.5	100.0
Feldspathic potassium	5.7	7.2	6.5	30.2
No potassium	2.8	4.2	3.5	16.3

Rape

Soluble potassium	8.2	8.1	8.2	100.0
Feldspathic potassium	2.7	3.2	3.0	36.6
No potassium	2.1	1.7	1.9	23.2

Buckwheat

Soluble potassium	5.9	6.8	6.4	100.0
Feldspathic potassium	2.6	2.5	2.6	40.6
No potassium	1.3	2.8	2.1	32.8

Alfalfa

Soluble potassium	3.0	3.9	3.5	100.0
Feldspathic potassium	4.1	4.2	4.2	120.0
No potassium	2.5	2.2	2.4	68.6

Alsike clover

Soluble potassium	5.1	4.0	4.6	100.0
Feldspathic potassium	4.3	4.5	4.4	95.7
No potassium	2.5	2.2	2.4	52.2

Peas

Soluble potassium	6.7	6.6	6.7	100.0
Feldspathic potassium	4.9	3.6	4.3	64.2
No potassium	4.4	4.2	4.3	64.2

Sorghum

Soluble potassium	30.0	31.0	30.5	100.0
Feldspathic potassium	7.0	5.8	6.4	21.0
No potassium	7.3	7.0	7.2	23.6

Sudan grass

Soluble potassium	13.8	11.5	12.7	100.0
Feldspathic potassium	4.3	3.3	3.8	29.9
No potassium	3.0	3.7	3.4	26.8

TABLE 1—*Concluded*

TREATMENT	YIELDS OF DRY TISSUE IN GRAMS			COMPARATIVE YIELDS
	A	B	Average	

1933 results—Concluded

Soybeans

Soluble potassium......................	8.2	8.7	8.5	100.0
Feldspathic potassium..................	5.3	5.0	5.2	61.2
No potassium..........................	5.8	4.9	5.4	63.5

Oats

Soluble potassium......................	8.7	7.2	8.0	100.0
Feldspathic potassium..................	3.7	3.5	3.6	45.0
No potassium..........................	2.8	3.0	2.9	36.3

Red clover

Soluble potassium......................	4.8	5.1	5.0	100.0
Feldspathic potassium..................	5.5	4.2	4.9	98.0
No potassium..........................	1.7	2.7	2.2	44.0

Sweet clover

Soluble potassium......................	5.2	4.7	5.0	100.0
Feldspathic potassium..................	6.3	6.2	6.3	126.0
No potassium..........................	3.3	2.5	2.9	58.0

1934 results

Buckwheat

Soluble potassium......................	13.3	11.0	12.2	100.0
Feldspathic potassium..................	2.4	1.4	1.9	15.6
No potassium..........................	1.5	0.9	1.2	9.8

Alfalfa

Soluble potassium......................	6.3	6.0	6.2	100.0
Feldspathic potassium..................	3.2	3.7	3.5	56.5
No potassium..........................	0.5	0.4	0.5	8.1

Sweet clover

Soluble potassium......................	6.3	5.8	6.1	100.0
Feldspathic potassium..................	3.3	3.3	3.3	54.1
No potassium..........................	0.6	0.7	0.7	11.3

and oats were poor feeders; whereas alfalfa, alsike clover, red clover, and sweet clover were relatively good feeders on feldspathic potassium.

The poor feeders grown on feldspathic potassium showed symptoms of potassium deficiency soon after the first leaves appeared. In the case of the good

feeders, however, there were no signs of potassium deficiency until the later stages of growth (112 days in 1933, and 87 days in 1934).

The 1933 crops of sweet clover and alfalfa grown on soluble potassium were not so good as desired. The reason for this lack of vigor is unknown. This condition made the relative feeding power of sweet clover and alfalfa for feldspathic potassium appear abnormally high. In view of this fact, alfalfa and sweet clover, along with buckwheat, were again tested in 1934, when sweet clover and alfalfa made a normal growth on soluble potassium, and their

TABLE 2

Potassium content of 12 different crops grown in quartz sand cultures supplied with soluble and feldspathic potassium

CROP	POTASSIUM CONTENTS OF CROPS GROWN WITH ADDITIONS INDICATED					
	Soluble potassium		Feldspathic potassium		No potassium	
	Per cent	Mgm. per jar	Per cent	Mgm. per jar	Per cent	Mgm. per jar
1933 results						
Peas...........................	1.66	111.22	0.28	12.04	0.23	9.89
Sorghum.......................	1.14	347.70	0.27	17.28	0.23	14.56
Sudan grass...................	1.94	246.38	0.35	13.30	0.35	11.90
Soybeans......................	3.04	258.40	0.72	37.44	0.71	38.34
Oats..........................	2.94	235.20	0.43	15.48	0.42	12.18
Corn.	1.73	371.95	0.43	27.95	0.41	14.35
Rape..........................	3.68	301.76	0.59	17.70	0.58	11.02
Buckwheat.....................	1.94	124.16	0.64	16.64	0.63	13.23
Alfalfa.......................	1.91	76.85	0.45	18.90	0.24	5.76
Alsike clover.................	2.04	93.84	0.42	18.48	0.24	5.76
Red clover....................	2.44	122.00	0.40	19.60	0.20	4.40
Sweet clover..................	2.12	106.00	0.44	27.72	0.23	6.67
1934 results						
Alfalfa.......................	1.61	99.82	0.41	14.35	0.50	4.25
Sweet clover..................	1.48	90.28	0.59	19.47	0.85	5.95
Buckwheat.....................	2.24	273.28	0.47	8.93	0.34	4.08

ability to feed on feldspathic potassium was confirmed. Buckwheat again exhibited poor feeding power for feldspathic potassium. The light conditions at Madison during the spring of 1934 were highly favorable to plant growth: more growth was obtained with sweet clover and alfalfa in 90 days in this season than in 120 days of the previous season. The normal growth on soluble potassium, together with the more rapid growth during this season, tended to reduce the apparent feeding power of alfalfa and sweet clover for feldspathic potassium. Perhaps the abnormal growth of the sweet clover and alfalfa during the spring of 1933 was due to the long periods of cloudiness that prevailed.

The potassium content of the alfalfa, alsike clover, red clover, and sweet clover crops grown on feldspathic potassium was significantly increased over that of the checks, as is shown in table 2. This is further evidence that these crops feed very markedly on the potassium in feldspar. The potassium content of the poor feeders, when grown on feldspathic potassium, did not show this significant increase.

Numerous theories regarding the feeding power of plants have been advanced. These are critically reviewed in a recent paper by Thomas (14). However, no theory has yet been advanced which adequately explains the differences observed in the feeding power of plants for feldspathic potassium.

Truog (15) suggested that good feeders obtain sufficient potassium from more dilute solutions than do the poor feeders. It appears from the work of previous investigators (2, 12) that plants do vary in their feeding power for potassium in dilute solution. In order to test this further, experiments were next conducted to determine whether the feeding power for feldspathic potassium was related to the capacity of a plant to obtain ample potassium from dilute solutions.

MINIMUM POTASSIUM CONCENTRATION NECESSARY FOR GOOD PLANT GROWTH

Methods

A study was made of the feeding power of buckwheat and red clover for potassium in dilute solution by means of flowing cultures. The culture vessels were 6-inch clay pots which had previously been heated and impregnated with paraffin. To each pot was added 2.5 kgm. of potassium-free sand. Sand cultures were used because they more closely approximate soil conditions as regards root development and aeration.

A nutrient solution, approximating in composition as regards the principal elements that found in the displaced solution of a very fertile soil, was prepared from potassium-free salts. It had the following composition:

Salts used, gm. per liter		Elements furnished, p.p.m.	
$Ca(NO_3)_2 \cdot 4H_2O$	0.5000	Calcium	160.0
$Ca(H_2PO_4)_2 \cdot 2H_2O$	0.0049	Magnesium	48.0
$CaCl_2 \cdot 2H_2O$	0.2756	Nitrogen	59.0
$MgSO_4 \cdot 7H_2O$	0.4866	Phosphorus	1.0
$MnCl_2 \cdot 4H_2O$	0.0004	Sulfur	63.0
NaI	0.00012	Chlorine	133.0
H_3BO_3	0.0006	Manganese	0.1
		Iodine	0.1
		Boron	0.1

About 2 liters of this solution percolated through each culture daily. Iron and additional phosphorus were supplied by adding and mixing 1.0 gm. $FePO_4$ with the sand of each culture. A potassium solution having a concentration of 1,000 p.p.m. of potassium was made by dissolving 1.9069 gm. of potassium chloride per liter. By adding from 0.5 to 7 cc. of this solution to each liter of

nutrient solution, potassium concentrations ranging from 0.5 to 7 p.p.m. of potassium were obtained.

The flow of the nutrient solution through the culture was controlled by the arrangement shown in figure 1.   Reservoir R is fitted with an air-tight stopper S, and is connected to the constant level jar C by means of an air siphon AA'
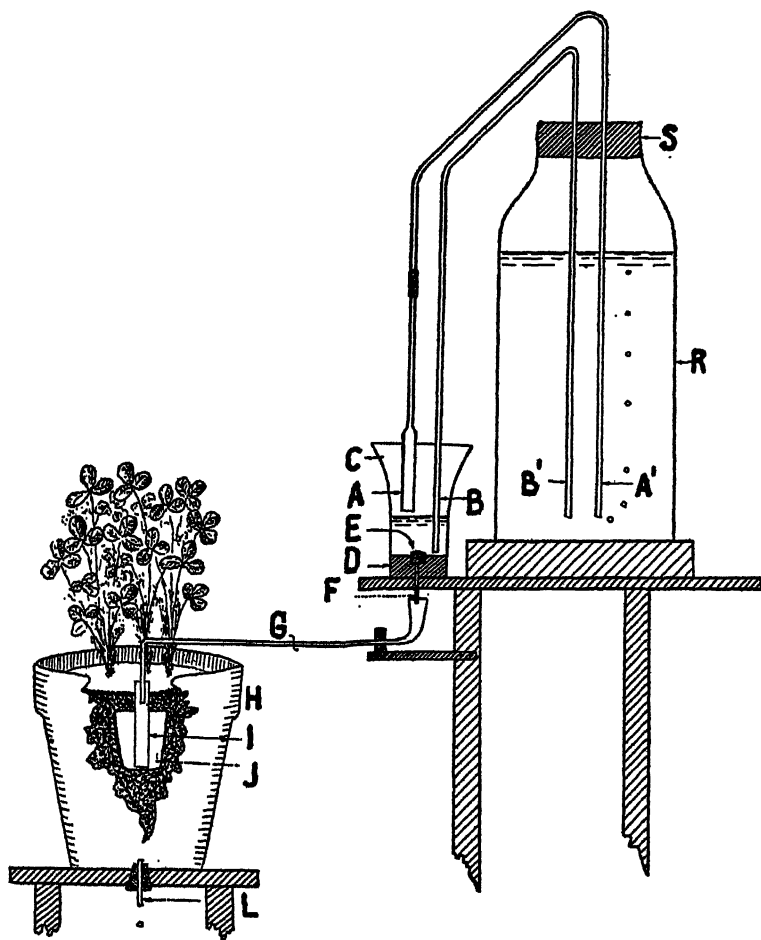
FIG. 1. APPARATUS FOR FLOWING CULTURE

and a water siphon BB'.   Outlet B should be at least an inch below intake B', and intake A should be near the level of outlet A'.   By extending the opening A' of the air siphon to the bottom of R, a more constant level is maintained in C than with an air siphon such as was used by Johnson (8).   A constant level is maintained in C by the alternate operation of the water siphon, which creates a partial vacuum in the reservoir, and the air siphon, which releases

this vacuum when the water level in C drops below A. Jar C is provided with a No. 8 one-hole rubber stopper D. A 0.2-mm. capillary tube F, 2 inches long, is inserted partly through D. The capillary tube, such as used by Allison and Shive (1) and by others, was found to need frequent cleaning. This was effectively overcome by filling the upper portion of D with cotton E, which filtered out any foreign material, and needed to be changed only occasionally. The same capillary may be used to deliver different amounts of nutrient solution by raising or lowering the level of A, which changes the pressure on F. If several percolators or pots are to be treated with the same solution, C is provided with the necessary number of capillaries. The nutrient solution is delivered through G to the pot H, where it drops into a glass tube I, and is conducted to the bottom of the crucible J, which is buried 1 inch below the level of the sand. The sand in J becomes saturated, and by capillary action and gravity the nutrient solution becomes distributed throughout the pot. Channeling by the nutrient solution is thus prevented, and there is continuous displacement of the solution as it percolates through the culture. Glass wool is placed over the tube L to prevent the escape of sand as the nutrient solution leaches out of the percolator. This apparatus is simple to operate, requires but little attention, and can easily be constructed from materials common in the average laboratory.

As soon as the plants came up, the flow of the nutrient solution was started. Ten days after planting, the plants were thinned to 8 per culture, care being taken to leave vigorous and well-distributed plants. The buckwheat was harvested after 35 days' growth, and the red clover, after 50. The potassium content of the dry tissue was determined as before. The results are given in table 3.

Results

The data indicate that the minimum potassium concentration necessary for good growth of buckwheat is close to 3 p.p.m. The plants receiving 1 p.p.m. potassium exhibited distinct symptoms of potassium starvation. Those receiving 2 p.p.m. appeared healthy, but had spindly stems and small leaves; 3 to 7 p.p.m. gave luxuriant growth. On the other hand, a potassium concentration of only 1 p.p.m. appears to suffice for red clover. The plants receiving 0.5 p.p.m. potassium showed symptoms of potassium starvation. It is evident that where good growth begins, the potassium content increases considerably. The work of previous investigators (2, 12) indicates that sweet clover and alfalfa make good growth at 0.5 p.p.m. of potassium, while oats, soybeans, sudan grass, etc. require from 2 to 3 p.p.m.

It appears, therefore, that good feeders on feldspathic potassium can obtain sufficient potassium from more dilute solution than the poor feeders. The question then arises: what special mechanism do strong feeders have which enables them to obtain ample potassium from dilute solution? The answer is unknown at the present time. It is to be noted that the good feeders are

plants which have a high potassium requirement. The data in table 2 likewise indicate that these crops have actually absorbed more potassium when grown on feldspathic potassium than have the poor feeders. It may be that the good feeders have an absorbing membrane through which the potassium diffuses more readily from dilute solution than through that of the poor feeders. This, of course, is purely hypothetical.

Perhaps a factor in this question is the rate of growth. Alfalfa and the clovers are plants which take considerable time to establish themselves. It is to be noted, the poor feeders are plants which normally grow very rapidly at the seedling stage. It seems reasonable that a crop which normally grows slowly at this early stage would utilize more advantageously the dilute solutions of potassium released by the slowly hydrolyzing feldspars. On the other hand, crops such as buckwheat, because of their rapid growth, would appear to require higher levels of available potassium.

TABLE 3

Yields and potassium contents of plants grown in quartz sand cultures supplied continuously with a nutrient solution containing the concentrations of potassium indicated

POTASSIUM IN NUTRIENT SOLUTION	DRY BUCKWHEAT TISSUE		DRY RED CLOVER TISSUE	
	Gm. per pot	Per cent potassium	Gm. per pot	Per cent potassium
p.p.m.				
0.0	1.2	0.11	0.3	0.55
0.5	...	....	0.4	0.68
1.0	2.5	0.78	1.0	1.11
2.0	4.0	0.76	1.6	1.24
3.0	5.5	1.02	1.5	1.53
4.0	5.5	1.15	...	....
5.0	5.7	1.14	1.3	1.97
7.0	6.2	1.30	...	....

PLANT FEEDING ON EXCHANGEABLE POTASSIUM

Bentonite is a naturally occurring clay having pronounced base exchange properties which are believed to be due to substances similar to those occurring in mineral soils. It was thus selected as one of the exchange substances for this experiment. It is well known that organic matter possesses base exchange properties. In peats and mucks, the major portions of the exchangeable bases are held in the organic exchange materials, consequently more information should be had on the availability of the exchangeable potassium in these materials.

Preparation of materials

Potassium-saturated bentonite was prepared in the following manner: Raw bentonite was leached with a solution N with respect to KCl and 0.05 N with respect to HCl. The purpose of the acid was to remove $CaCO_3$. Leaching

was continued until the leachate gave no further test for calcium. The bentonite was then leached with a large volume of N KCl solution to saturate the bentonite with potassium. It was then washed with 85 per cent, by volume, alcohol until the leachate was chloride-free. The bentonite was partially dried, dispersed in water, and the suspension diluted to a large volume and permitted to stand overnight, so as to allow the coarser materials to settle. The supernatant suspension was siphoned off, evaporated to dryness, and the residue ground to pass a 100-mesh sieve. Analysis showed this material to be 93 per cent saturated with potassium. It had a pH of about 7.2.

Half-saturated potassium bentonite was prepared by treating hydrogen-saturated bentonite with K_2CO_3. Hydrogen-saturated bentonite was prepared in the following manner: Raw bentonite was dispersed in water, and the supernatant suspension was siphoned off and evaporated to dryness; the residue was ground to pass a 40-mesh sieve and leached with a solution N with respect to NH_4NO_3 and 0.05 N with respect to HNO_3. The NH_4NO_3 prevented excessive swelling of the bentonite. When the leachate was calcium-free, the excess NH_4NO_3 and HNO_3 were washed out with 85 per cent, by volume, alcohol, and the bentonite was dried and powdered. The remaining NH_4NO_3, HNO_3, and exchangeable NH_3 were driven off by ignition at 450°C. The hydrogen-saturated bentonite thus prepared was found to be NH_3-free. Sufficient potassium as a dilute solution of K_2CO_3 was added to just half-saturate the bentonite. After evaporation to dryness, the bentonite was ground to pass a 100-mesh sieve. It had a pH of about 5.7.

Saturated and half-saturated potassium-organic exchange materials were prepared in the following manner: Raw peat was leached with 0.05 N HNO_3 until free of calcium. The excess HNO_3 was washed out with distilled water, and the peat was dried. Two portions of this hydrogen-saturated material, the total exchange capacity of which had been determined by the usual method, were weighed out, and, to the first, sufficient potassium was added as a dilute K_2CO_3 solution just to saturate the exchange material, and to the second, just sufficient for one-half saturation. The mixtures were evaporated to dryness, and the organic exchange material was ground to pass a 100-mesh sieve.

Methods of plant culture

To prevent replacement of the exchangeable potassium by the bases of the other nutrients, Prianischnokov's (13) isolation method was used in which the exchange materials are placed in an inner jar so as to prevent contact with the nutrient solution, and part of the root system of each plant is looped into this jar so that it may feed on exchangeable potassium.

Potassium in the form of saturated and half-saturated potassium inorganic and organic exchange material and as KCl was added in all cases at the rate of 100 pounds per acre of elemental potassium. The treatments were tested in some cases in duplicate and in others in triplicate. The KCl was dissolved and added with the nutrient solution. Salts free of potassium were used for

supplying the other nutrients. Phosphorus was added to the sand of the outer jar in the manner described under the feldspar experiments. Three nutrient solutions were prepared to supply the other essential elements, namely:

(A) 50 gm. $Ca(NO_3)_2 \cdot 4H_2O$
     Sufficient H_2O to give 1,000 cc. after solution

(B1) 26 gm. $MgSO_4 \cdot 7H_2O$
     27 gm. $Mg(NO_3)_2 \cdot 6H_2O$
     0.15 gm. $MnCl_2 \cdot 4H_2O$
     0.05 gm. NaI
     Sufficient H_2O to give 1,000 cc. after solution

(B2) 13 gm. $MgSO_4 \cdot 7H_2O$
     14 gm. $Mg(NO_3)_2 \cdot 6H_2O$
     0.15 gm. $MnCl_2 \cdot 4H_2O$
     0.05 gm. NaI
     Sufficient H_2O to give 1,000 cc. after solution.

Twenty cubic centimeters per pot of solutions A and B1 were added to corn, oats, and sudan grass. A nutrient solution having a higher Ca-Mg ratio (3 to 1) was supplied to the alfalfa as follows: Twenty cubic centimeters of solutions A and B2 were added to each jar, and additional calcium to increase the Ca-Mg ratio was supplied by mixing with the sand of the outer jar 0.26 gm. $CaSO_4$. The cultures were also inoculated. The potassium exchange material, mixed with some sand, was put into the inner jar, which was a glass vessel 4 inches in diameter and 5 inches in depth. The inner jar was then placed in the 2-gallon jar, the nutrient solutions were added and mixed with about 500 cc. of water, and the diluted solution was carefully added to the sand in the outside container.

Ten-day-old plants were transplanted to the jars, the root system of each plant being looped into the inner jar so it might feed on the exchangeable potassium. Sufficient moist sand, free of salts, was added to cover the roots of the plants. After 7 days, the corn was thinned to 3 plants per jar, and the other crops to 10. Care was taken to leave vigorous and well-distributed plants in each jar. After 45 to 60 days growth, the corn, sudan grass, and oats were harvested. The alfalfa was harvested after 120 days' growth. The tissue was dried at 75° to 80°C. and weighed; replicates were composited, ground, and in some cases analyzed for potassium as before. The results are given in tables 4 and 5, and figure 2 of plate 1 illustrates the results with sudan grass.

Results

In calculating the comparative yields in table 4, the yields obtained with soluble potassium are taken as a standard and expressed by 100, and the other yields are expressed as percentages of this. The data in table 4 show that the potassium held in organic and inorganic exchange materials is available to plants and that the availability of exchangeable potassium decreases with decreasing degree of potassium saturation of the exchange materials. Because

of the precautions taken to prevent replacement of potassium by the bases of other nutrients, it is evident that plants can feed directly on exchangeable

TABLE 4

Yields of oven-dry material of different crops grown in quartz sand cultures supplied with soluble and exchangeable potassium

TREATMENT	YIELDS IN GRAMS						AVERAGE YIELD TOPS AND ROOTS	COMPARATIVE YIELDS
	A		B		C			
	Tops	Roots	Tops	Roots	Tops	Roots		
Alfalfa								
Soluble K*...................................	3.8	1.5	3.8	2.1	4.0	2.4	5.9	100.0
K-saturated inorganic exchange material.......	4.5	2.6	5.3	2.9	5.4	2.0	7.6	128.8
Half-K-saturated inorganic exchange material...	4.2	1.9	5.0	2.3	5.2	2.8	7.1	120.4
No K.......................................	2.4	0.9	2.0	1.2	1.5	0.5	2.8	47.5
Oats								
Soluble K...................................	12.0	4.9	10.4	3.2	11.3	3.0	14.9	100.0
K-saturated inorganic exchange material.......	11.2	2.5	10.0	4.8	10.2	3.5	14.1	94.6
Half-K-saturated inorganic exchange material...	10.0	3.5	9.5	2.7	10.2	3.3	13.0	87.2
No K.......................................	6.3	1.5	6.0	2.2	5.8	2.5	8.1	54.4
Sudan grass								
Soluble K...................................	11.5	13.5	8.7	13.0	....	....	23.4	100.0
K-saturated inorganic exchange material.......	6.3	12.7	7.5	9.5	....	....	18.0	76.9
Half-K-saturated inorganic exchange material...	3.8	5.3	4.6	6.1	....	....	9.9	42.3
No K.......................................	1.3	2.3	1.5	3.2	....	....	4.2	17.9
Corn								
Soluble K...................................	16.4	8.5	17.0	9.7	....	....	25.8	100.0
K-saturated inorganic exchange material.......	14.9	10.3	10.3	13.2	....	....	24.4	94.6
Half-K-saturated inorganic exchange material...	14.3	8.0	15.2	6.3	....	....	21.9	84.9
No K.......................................	4.5	2.8	4.3	6.0	....	....	8.8	34.1
Corn								
Soluble K + H-saturated organic exchange material...................................	14.0	11.3	14.3	14.2	....	....	26.9	100.0
K-saturated organic exchange material..........	12.8	13.7	13.5	13.3	....	....	26.7	99.3
Half-K-saturated organic exchange material....	12.5	10.0	8.7	6.3	....	....	18.8	70.0
No K + H-saturated organic exchange material..	2.8	1.7	3.3	3.5	....	....	5.7	21.2

* All replicates of this treatment with alfalfa damaged by mice.

potassium by exchanging the hydrogen ion of the carbonic acid secreted by their roots for the potassium in the exchange materials. Jenny and Cowan (7) report similar results for calcium.

The data in table 5 show that the plants absorbed considerable amounts of potassium from the exchange materials. There was a slight increase in the content of potassium in the crops grown on saturated potassium bentonite over those grown on half-saturated bentonite.

NATURE OF POTASSIUM IN SAP OF PLANTS

Our knowledge concerning the feeding power of plants for potassium would probably be somewhat clarified if more information concerning the nature of the potassium in the sap of plants, particularly as regards its solubility, were at hand. Kostytschew and Eliasberg (9) extracting dry tissue with water, Janssen and Bartholomew (6) microchemically precipitating the potassium in plant cells, and Morris (11) extracting fresh tissue with hot water, report that the potassium compounds in plants are in solution or in readily soluble forms. There is always the possibility that the treatments which they gave the plant tissue may have changed the potassium to a more soluble form. These treat-

TABLE 5

Potassium content of some crops grown in quartz sand supplied with soluble and inorganic exchange potassium

CROP	PER CENT POTASSIUM IN OVEN-DRY TISSUE PRODUCED WITH POTASSIUM ADDITIONS INDICATED			
	Soluble potassium	Saturated potassium bentonite	Half-saturated potassium bentonite	No potassium
Oats.................................	2.25	0.69	0.67	0.32
Alfalfa...............................	2.20	1.61	1.59	0.67
Sudan grass...........................	2.21	1.28	1.26	0.44

ments can be avoided by dialyzing the expressed sap of plants. From the slope of the dialysis rate curve, some idea may be obtained of whether or not the potassium in plant tissue is in solution. It is true that the tissue is killed by macerating and expressing the sap, but it is believed that this is the best available method for attacking the problem.

Dialysis of expressed sap

Fresh samples of rape, sweet clover, alfalfa, buckwheat, corn, and soybeans growing in the vicinity of Madison were collected and immediately placed in a jar and covered with cracked ice. On arrival at the laboratory, the tissue was ground and the sap immediately expressed with a pressure of 6,000 pounds per square inch for 5 minutes. Two 10-cc. aliquots of the expressed sap were taken, the first for total potassium analysis, and the second for dialysis. The latter was placed in a collodion bag which was hung in a beaker containing 500 cc. of water at 2° to 5°C. so that the potassium could dialyze out of the sap into the water. The low temperature minimized enzymatic activity during

dialysis. The water surrounding the collodion bag was stirred continuously for 3 hours, and the dialyzate was sampled regularly for potassium analysis. After the third hour, stirring was stopped, and the beaker with its contents was placed overnight in a refrigerator to come to final equilibrium. The results, which are given in tables 6 and 7 and figure 2, indicate that the potas-
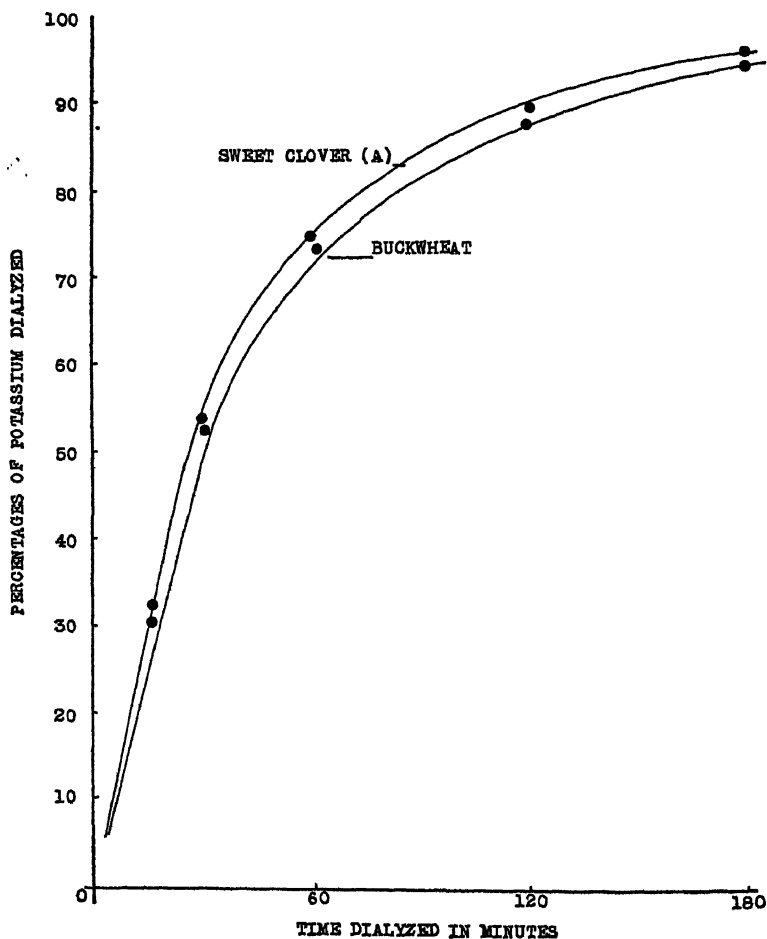
FIG. 2. RATE OF DIALYSIS OF POTASSIUM FROM THE EXPRESSED SAP OF SWEET CLOVER AND BUCKWHEAT THROUGH A COLLODION MEMBRANE

sium in the expressed sap of plants is practically all in dialyzable form. There is no significant difference in the rate of dialysis of potassium from the sap of various plants; this indicates a similarity of form.

The question arose as to how the plant sap dialysis curve would compare with that of a solution of a potassium salt. A KCl solution having the same

potassium concentration as the sap of sweet clover (A) was prepared.   A 10-cc. aliquot of this solution was dialyzed under the same conditions as the plant saps.   The results are plotted in figure 3.   The curve shows a marked similarity in the slope and shape to that for the sap of sweet clover (A).   This is evidence that the potassium in the expressed sap of sweet clover is practically all in solution.   It was suspected that the slight lag in the rate of dialysis

TABLE 6

The total and dialyzable potassium in the expressed sap of different plants

PLANT	POTASSIUM IN 10 CC. EXPRESSED SAP	POTASSIUM REMOVED FROM 10 CC. EXPRESSED SAP BY DIALYSIS	DIALYZABLE POTASSIUM
	mgm.	*mgm.*	*per cent*
Rape..	30.96	29.36	94.9
Sweet clover (A)................................	36.32	34.86	96.0
Sweet clover...................................	36.37	34.63	95.2
Buckwheat.....................................	35.72	34.32	96.1
Alfalfa..	61.92	59.90	96.7
Alfalfa..	62.57	58.45	93.4
Corn..	26.20	25.27	96.5
Soybeans......................................	54.43	52.37	96.2

TABLE 7

The rate of dialysis of the potassium in the expressed sap of different plants through a collodion membrane

PERCENTAGES OF POTASSIUM DIALYZED IN PERIODS INDICATED

	15 minutes	30 minutes	60 minutes	120 minutes	180 minutes
Rape............................	30.0	50.8	69.9	85.8	92.5
Sweet clover (A)...................	32.7	54.0	74.4	89.5	96.4
Sweet clover.....................	31.7	50.4	68.1	81.5	90.1
Buckwheat.......................	30.4	52.5	75.1	87.2	94.6
Alfalfa..........................	43.5	56.3	74.8	85.0	95.5
Alfalfa..........................	33.4	44.6	67.0	82.0	87.2
Corn............................	43.4	59.9	84.2	98.3	99.3
Soybeans........................	33.0	54.9	67.5	87.4	92.2

of potassium from the sweet clover sap was due to suspended matter—chloroplasts, protein colloids, etc.   In order to test this point, an amount of potassium equal to that in the sweet clover sap was added to a thick suspension of purified $Fe(OH)_3$, and dialysis was carried out as previously described.   The curve plotted in figure 3 shows that suspended matter very markedly slows down the rate of dialysis of soluble salts and that the lag in the rate of dialysis of sweet clover sap was probably due to the suspended matter present.
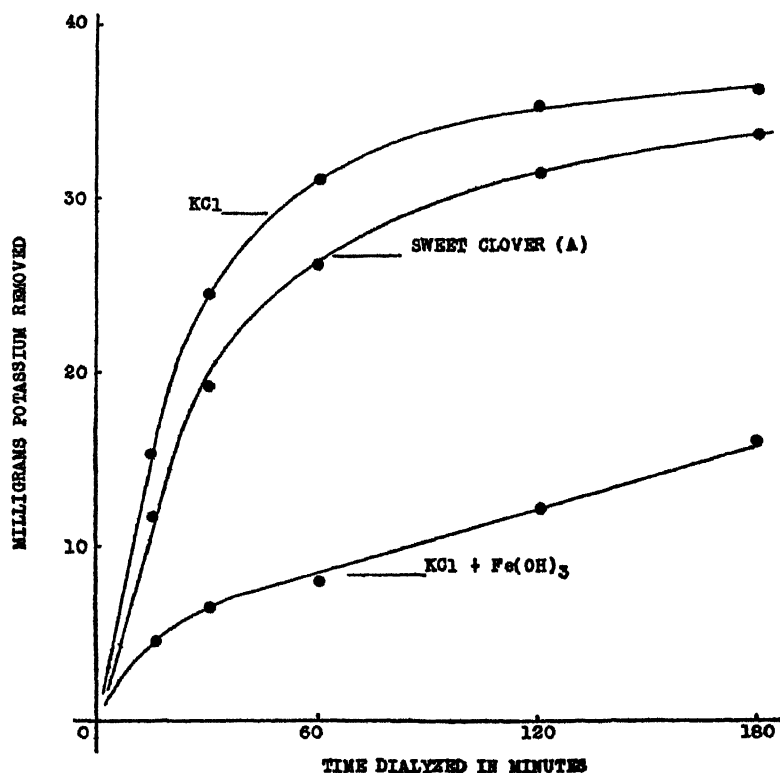
FIG. 3. RATE OF DIALYSIS OF POTASSIUM FROM SWEET CLOVER SAP COMPARED TO KCl SOLUTIONS CONTAINING EQUAL CONCENTRATIONS OF POTASSIUM, ONE WITH AND ONE WITHOUT Fe(OH)₃

TABLE 8

The total and dialyzable potassium in macerated bluegrass and sweet clover tissue

PLANT	TOTAL POTASSIUM IN TISSUE	POTASSIUM REMOVED FROM TISSUE BY DIALYSIS	DIALYZABLE POTASSIUM
	mgm.	*mgm.*	*per cent*
Bluegrass	74.59	73.50	98.5
Bluegrass	51.81	50.91	98.3
Sweet clover	38.63	38.15	98.8
Sweet clover	41.38	40.62	98.2

Dialysis of macerated tissue

To determine whether all the potassium in a plant is in solution or in a readily soluble form, macerated tissues of sweet clover and bluegrass, in place of the expressed sap previously used, were put into collodion bags which were hung in beakers of water and dialyzed at 2° to 5°C. The water in the beakers was

changed frequently. When the dialyzate gave no further test for potassium, the collodion bags and the macerated tissues were dried and ashed, and the potassium contents were determined. The results are given in table 8, and show that the potassium in macerated sweet clover and bluegrass tissues is practically all in a dialyzable form and hence in solution or readily soluble form.

Relation of results to plant feeding

Since the potassium in different plants seems to be very similar as regards solubility and form, it appears that the differences in feeding power of different plants are not related to any internal condition of the potassium and that these differences in feeding power will have to be explained on some other basis.

SUMMARY

The purpose of this investigation was to gain further information about the feeding power of plants for feldspathic and exchangeable potassium, and why there are differences in the feeding power of plants for potassium. The plants were grown in a greenhouse in quartz sand cultures, to which were applied: (a) feldspathic potassium, (b) exchangeable potassium, and (c) dilute solutions of water-soluble potassium. A study of the nature of the potassium in the expressed sap of plants was also made. The results of the investigation are as follows:

Corn, rape, buckwheat, peas, sorghum, sudan grass, soybeans, and oats were poor feeders on feldspathic potassium, whereas alfalfa, alsike clover, red clover, and sweet clover were relatively good feeders.

The potassium content of the alfalfa, alsike clover, red clover, and sweet clover plants receiving feldspathic potassium was significantly increased over those not receiving potassium, whereas that of the poor feeders did not show this significant increase.

Flowing cultures showed that the minimum concentration of potassium necessary for good growth of buckwheat is close to 3 p.p.m., but that about 1 p.p.m. suffices for good growth of red clover.

The feeding power of plants for the potassium of feldspars appears to be dependent on their ability to utilize more advantageously the potassium in dilute solution, which, in turn, may depend partly on the rate of plant growth.

The potassium in inorganic and organic exchange form is very readily available. Its availability decreases with a decreasing degree of potassium saturation of the exchange materials. Plant roots, by secreting carbonic acid, can feed directly on exchangeable potassium.

There is no significant difference in the rate of dialysis of potassium from the expressed sap of rape, sweet clover, buckwheat, alfalfa, corn, and soybeans; this indicates a similarity of form. The potassium in the macerated tissue of bluegrass and sweet clover is practically all in dialyzable form, and hence is in solution or in a state which readily hydrolyzes to a soluble form on dialysis. Consequently, the differences in feeding power are probably not related to any internal condition of the potassium.

The construction and operation of an apparatus for flowing cultures, which has several desirable features, is described.

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PLATE 1

GROWTH OF SWEET CLOVER AND SUDAN GRASS WITH VARIOUS POTASSIUM TREATMENTS

FIG. 1. Growth of sweet clover with the potassium treatments indicated.   Jar 1, 100 pounds per acre soluble potassium; jar 2, 2 tons per acre feldspar; jar 3, no potassium.

FIG. 2. Growth of sudan grass with potassium treatments indicated.   Jar 1, 100 pounds soluble potassium per acre; jar 2, 100 pounds potassium per acre as saturated inorganic exchange material; jar 3, 100 pounds potassium per acre as half-saturated inorganic exchange material; jar 4, no potassium.

TOLERANCE OF CERTAIN WEEDS AND GRASSES TO TOXIC ALUMINUM[1]

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In 1905 a series of experiments with lawn grasses was begun at the Rhode Island Agricultural Experiment Station. In several reports published since that time evidence has been given to show that the prevalence of weeds is less where the acidity of the soil is high. Although theories have been given to explain such a phenomenon, no facts have been developed to substantiate them.

Several workers at the Rhode Island station (3) and elsewhere have produced experimental evidence to show that soil acidity with its resultant depressing action upon plant growth is linked with toxic aluminum set free in the soil solution. Further attempts to clarify the picture have resulted in assigning to various crop plants (2) tolerance ranges within which certain quantities of aluminum fed to plants in solution cultures have been found to be toxic.

When we connect the knowledge of the lower prevalence of weeds on acid lawn soils and the relative sensitivity of crop plants it seems reasonable to suppose that weeds in general may have certain tolerance ranges and that individual species may vary as to their resistance to toxic aluminum. Such an assumption has led to an experiment to test the relative susceptibility of weed species commonly found in competition in lawn grass stands, and also, by way of comparison, the amounts of aluminum necessary to inhibit growth of some of the common lawn grasses have also been determined.

PROCEDURE

Seeds of the following weeds and grasses were collected and germinated in sand-soil mixtures in flats in the greenhouse.

1. *Cerastium vulgatum*.................................Mouse-ear chickweed
2. *Stellaria media*.......................................Common chickweed
3. *Taraxacum officinale*...............................Common dandelion
4. *Leontodon autumnalis*.............................Fall dandelion
5. *Digitaria humifusa*..................................Smooth crabgrass
6. *Digitaria sanguinalis*..............................Rough crabgrass
7. *Prunella vulgaris*...................................Heal All
8. *Setaria glauca*......................................Yellow Foxtail
9. *Poa pratensis*.......................................Kentucky bluegrass
10. *Agrostis tenuis*....................................R. I. (Colonial) Bent
11. *Agrostis alba*......................................Redtop

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425

When the root systems had become established the soil was washed off the roots and the plants were mounted in perforated paraffined corks, six plants to each cork, and placed in 250-cc. bottles of nutrient solution. The basic nutrient solution used was a modification of Rhode Island solution A (1) having the following ingredients in 1 liter of solution.

Ingredients	cc.	Molar
$Ca(NO_3)_2 \cdot 4H_2O$	15	0.100
$MgSO_4 \cdot 7H_2O$	8	0.100
$Fe_2(NO_3)_6 \cdot 18H_2O$	4	0.003
KCl	8	0.100
$CaH_4(PO_4)_2H_2O$	8	0.010
NH_4NO_3	10	0.100

At all times and in all experiments the controls were grown in the solution given as above. The aluminum treatment was provided by using the alternating solution method adopted by McLean and Gilbert (2). By this method the plants were grown in solution A from Monday to Thursday of each week. They were then changed to a culture solution containing no phosphorus but having added to it the desired strength of aluminum in the form of aluminum sulfate. The acidity of certain of the control solutions was adjusted to that of the aluminum culture (pH 3.6–5.0) for additional proof that active acidity *per se* is not the cause of depressed growth.

<center>PRESENTATION OF DATA AND DISCUSSION</center>

In table 1 are given relative yields which show in a comparative way the sensitivity of the various plants studied. For greater ease of comparison the plants have been arranged in three groups according to their aluminum sensitivity, as follows:

Sensitive (depression 20 + per cent with 2–8 p.p.m. aluminum)
  Mouse-ear chickweed, common chickweed, common dandelion, yellow foxtail, Heal All, and Kentucky bluegrass.

Medium Sensitive (depression 20 + per cent with 16–32 p.p.m. aluminum)
  Fall dandelion and smooth crabgrass.

Resistant (depression 20 + per cent with 32–80 p.p.m. aluminum)
  Rough crabgrass, redtop, and colonial bent.

It will be noted that Colonial bent and redtop required much higher concentrations to produce a depressed growth than did bluegrass. This is, of course, in line with the long-observed facts with regard to the growth of these plants on acid soils.

Observations of a more or less indefinite nature which have been made at the Rhode Island station from time to time have pointed to differences in the persistence of various weeds when under the same competition as to stand. Crabgrass is well-known as a persistent weed, whereas chickweed can be largely controlled by using sulfate of ammonia and by rendering the soil acid. These observations are in agreement with the ranges of sensitivity as given in table

TABLE 1

Relative yields of plants as affected by quantities of aluminum in solution

PLANT	SERIES	CHECK	2 p.p.m. Al	4 p.p.m. Al	8 p.p.m. Al	16 p.p.m. Al	24 p.p.m. Al	32 p.p.m. Al	40 p.p.m. Al	60 p.p.m. Al	80 p.p.m. Al	Sulfuric acid
							RELATIVE YIELDS OF PLANTS WITH TREATMENTS OF					
Group I. Depression 20+ per cent with 2–8 p.p.m. Aluminum												
Mouse-ear chickweed	I	100	62.0	54.8	56.5*	......	27.8	....	....	15.7	....	91.3
	II	100	......	66.9*	49.7	29.9	19.7	....	....	....	....	98.7
	III	100	......	......	64.7*	......	....	....	....	....	....	101.9
Common dandelion	I	100	......	......	49.3*	....	....	....	....	....	....	84.0
	II	100	29.0*	20.4	17.2	...:	....	....	....	....	....	87.3
	III	100	37.9	20.4	21.4*	....	....	....	....	....	....	102.9
	IV	100	......	70.3*	....	....	....	....	....	....	....	106.8
Kentucky bluegrass	I	100	74.4*	88.4	33.9	....	....	....	....	....	....	114.0
	II	100	......	52.8*	41.5	49.0	....	....	....	....	....	94.3
Common chickweed	I	100	78.1	......	46.1	31.3*	34.4	....	....	26.6	....	111.7
	II	100	98.5	......	64.4*	......	52.3	....	....	28.8	....	126.5
	III	100	......	......	80.4*	....	....	....	....	....	....	96.4
Heal all	I	100	......	......	50.0*	....	....	....	....	....	....	84.6
	II	100	......	62.4	50.0	39.3	24.5*	....	....	....	....	108.0
Group II. Depression 20+ per cent with 16–32 p.p.m. Aluminum												
Fall dandelion	I	100	......	......	100.8*	......	....	....	....	....	....	142.1
	II	100	......	......	69.6	60.3*	47.6	....	....	11.8	....	96.9
	III	100	105.4	......	83.1*	......	54.7	....	....	28.4	....	98.0
	IV	100	......	......	......	60.0*	....	....	....	....	....	109.0
	V	100	......	......	73.7*	76.8	68.4	76.8	....	....	....	83.7
Smooth crabgrass	I	100	......	......	105.5*	93.0	82.5	75.2	....	....	....	113.0
	II	100	......	......	......	100.0*	97.5	....	....	....	....	101.1
	III	100	......	......	......	85.0*	75.2	....	76.0	....	....	65.9
	IV	100	......	......	......	......	97.0	....	94.1	....	....	100.0
	V	100	86.8	79.2	68.1*	......	70.1	....	74.3	79.2	....	77.0
	VI	100	87.8	113.4	131.7	112.2	80.5*	....	76.8	75.6	....	.....
Group III. Depression 20+ per cent with 32–80 p.p.m. Aluminum												
R. I. bent	I	100	......	89.0*	74.3	86.8	97.1	....	....	....	....	130.1
	II	100	......	92.7*	127.6	84.5	84.5	....	....	....	....	87.9
Redtop	I	100	......	117.2*	91.4	94.8	97.4	....	....	....	....	135.3
Rough crabgrass	I	100	......	......	......	......	84.9*	....	90.9	....	....	71.5
	II	100	......	......	......	......	71.9*	....	73.4	....	....	74.0
	III	100	132.5	82.5	106.1	88.6	144.7	....	89.5	129.8	97.4	.....
	IV	100	......	......	......	......	97.0*	....	94.0	....	....	100.0

* The asterisk designates the culture in each series having a pH comparable to the corresponding sulfuric acid culture.

427

1, crabgrass being very resistant and chickweed very sensitive to toxic aluminum.

As in former experiments (2) where the toxic effects of aluminum have seemed to be localized in the roots and evidenced by abnormal root growth, so with these weed species the root development was quite abnormal. Roots in the higher aluminum concentrations were brown in color with fewer rootlets and had definitely discolored root tips. That plants grown at acidities arranged by the use of H_2SO_4, and similar to the aluminum treatments, were not depressed in growth is shown by plate 1. Here common chickweed (*Stellaria media*) is shown with four treatments: check culture solution; with 8 p.p.m. of aluminum; with 60 p.p.m. aluminum; and with a solution adjusted to a comparable acidity by use of H_2SO_4. The relative yields of various plants with acidities so adjusted are shown in table 1. It is thus quite apparent that, as has been found in former experiments (1) with solution cultures, weeds grown at high acidities are not affected by acidity *per se*.

Finally it is suggested that, since there are such wide differences in sensitivity between the various weed species, the control of weeds in a competitive grass stand where soil acidity is maintained may be due, in part, to toxic aluminum. During the early stages of germination the newly formed tissues are soft and delicate and may be readily susceptible to aluminum toxicity. In this stage of plant development chickweed seedlings, being sensitive to small amounts of aluminum, might easily be killed. On the other hand, seedlings of crabgrass might survive under concentrations of aluminum which would be fatal to the chickweed seedlings.

SUMMARY

Experimental evidence is given to show that the various weed species commonly found in competition with lawn grasses vary greatly in their sensitivity to aluminum in solution cultures. From these facts it is suggested that toxic aluminum in the soil solution may be one of the causes of the inhibition of weed growth on acid soils.

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PLATE 1

EFFECT OF ALUMINUM ON COMMON CHICKWEED (*Stellaria media*)

I. Check culture solution.
II. 8 p.p.m. aluminum.
III. 60 p.p.m. aluminum.
IV. Check culture solution adjusted to comparable acidity with aluminum treatments by use of sulfuric acid.

BASIL E. GILBERT AND FREDERICK R. PEMBER

RATES OF ABSORPTION OF AMMONIUM AND NITRATE NITROGEN FROM CULTURE SOLUTIONS BY TEN-DAY-OLD TOMATO SEEDLINGS AT TWO pH LEVELS[1]

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Considerable work has hitherto been published on the rates of absorption of ammonium and nitrate nitrogen by different species of plants from culture solutions, under various conditions of growth. It has been shown that, under similar experimental conditions, these absorption rates vary significantly for a given species at different stages during the growth cycle. It has also been shown that plants exhibit a very pronounced species difference with respect to the absorption and assimilation of nitrogen in the cation and anion forms.

For example, Stahl and Shive (6, 7), working with oats and buckwheat, found a very distinct contrast between these two species in their absorption rates of ammonium and nitrate nitrogen. With oats, the rate of ammonium-nitrogen absorption was greater than that of nitrate-nitrogen during the early stages of growth, but during the later stages the situation was reversed. During the greater part of the life cycle of this species, nitrate-nitrogen absorption predominated over ammonium-nitrogen absorption, both in actual quantity absorbed and in rate, or quantity, absorbed per unit of plant tissue, when both forms were present simultaneously in the growth medium at approximately equal concentrations.

With buckwheat, on the contrary, ammonium-nitrogen absorption predominated over nitrate-nitrogen absorption during the greater part of the active life cycle of the plant. The nitrate absorption rate exceeded the ammonium absorption rate only in the very late stages of growth when the absorption rates of both were relatively low. Thus, the buckwheat may be spoken of as an "ammonium absorber," in contrast to the oat plant, which may be considered as a "nitrate absorber."

More recent work by Clark and Shive (1) with the tomato plant has shown that during the middle and later stages of growth this species shows a close parallel to the oat plant in its tendency toward nitrate absorption, as predominant over ammonium; but Clark and Shive did not determine the rates of nitrogen absorption by this plant at a very early stage of development. It is the purpose here to present briefly the results of tests made with tomato

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seedlings at as early a developmental stage as was experimentally practicable, and thus to fill in this rather important gap left vacant in their work.

METHODS OF EXPERIMENTATION

The plants used for this test were young seedlings 10 days old from germination. The first foliage leaf of each seedling was just beginning to enlarge when these tests were made. The seeds were germinated and the seedlings grown by the same general methods, with slight modifications, as were used and described by Stahl and Shive (7) in similar work with buckwheat seedlings. After germination between moist blotting papers, the seeds were transferred to paraffined cloth nets supported on shallow glass dishes, each dish containing approximately 400 cc. of culture solution. On each net, 250 germinated seeds were placed.

TABLE 1

Composition of solutions used

Basic solution at a concentration of 1 atmosphere

SOLUTION DESIGNATION	PARTIAL VOLUME-MOLECULAR CONCENTRATIONS			
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	$(NH_4)_2SO_4$
Modified Tottingham............				
$T_3R_2C_2$.........................	0.00211	0.0029	0.0071	0.0028

Modifications		
QUANTITY	ACID AND ALKALI PER LITER	RESULTING pH
cc.		
0.14	Normal H_2SO_4	4.0
2.25	Normal KOH	7.0

The solution used for growing and testing these seedlings was the $T_3R_2C_2$ solution of the four-salt Tottingham type, modified by Jones and Shive (3) to contain ammonium sulfate, and was identical with the culture solution employed by Clark and Shive (1). This particular solution contains approximately equal molar concentrations of ammonium and nitrate nitrogen, and was here further modified, as was also done by Clark and Shive (1), to contain only one-third as much monopotassium phosphate as the original solution, in order to lessen the tendency toward precipitation of the phosphate ion in combination with the other ions in the final solution when employed at pH 7.0.

For the first 2 days after the germinated seeds had been transferred to the nets, the solution was supplied continuously to the seedlings at one-tenth atmosphere osmotic concentration. For the following 3 days it was then supplied at one-quarter atmosphere, after which time the plants were grown and tested in the solution at approximately eight-tenths atmosphere. All the cultures were grown, up to the time of testing, in the culture solution at the

same pH level: pH 5.3.   In view, however, of the recent work by Clark and
Shive (1), who showed that a significant relationship exists between the pH of
the culture solution and the rates of absorption of the two forms of nitrogen,
the tests were made at two different pH levels: pH 4.0 and pH 7.0.   The quan-
tities of salts, acid, and alkali used in making up this solution at the final con-
centrations and at the pH levels used are given in table 1.   Boron, man-
ganese, and iron, in the forms of boric acid, manganese sulfate, and ferrous
sulfate, respectively, and in quantities of 0.5 p.p.m. of each, were added to all
solutions used.

The tests for absorption rates were carried out during the middle of the day,
under conditions of bright sunlight and active transpiration.   The absorption
interval extended over a period of 6 hours, during which time the roots of the
seedlings were immersed in 350 cc. of the culture solution that had been
measured into the rinsed containers at the beginning of the test period.   Dur-
ing the absorption interval of 6 hours, 250 cc. additional solution was dripped
through each culture vessel by the continuous flow method used and described
by Shive and Stahl (5).

At the end of the absorption interval, the container of each culture was
carefully emptied and, together with the net and roots, rinsed clean of solution.
The test solution and rinse water were combined and made up to a volume of 1
liter.   The analyses of the test solutions for the two forms of nitrogen, both
before and after contact with the roots of the seedlings during the test intervals,
were carried out by the methods used and described by Clark and Shive (1),
and the amounts of nitrogen absorbed by the seedlings were calculated by
difference.

<center>EXPERIMENTAL RESULTS</center>

The absorption data for these young seedlings are presented in table 2.   All
values are calculated in terms of milligrams of nitrogen absorbed per 10 gm.
of green weight of plant tissue in 6 hours.

The first point which stands out clearly is the fact that for these young
tomato seedlings, under the experimental conditions here defined, the rates of
absorption of nitrate nitrogen greatly exceed the rates of absorption of am-
monium nitrogen at both high and low pH levels.   Direct comparison shows
the average nitrogen-anion absorption rate values to be 4.7 and 1.2 times the
corresponding cation-nitrogen absorption rate values at pH 4.0 and pH 7.0
respectively.   This is in direct contrast with the results obtained by Jones
and Skinner (2) for soybeans and corn, by Naftel (4) for cotton, and by Stahl
and Shive (6, 7) for oats and buckwheat.

In considering now the ammonium- and nitrate-nitrogen absorption rate
values separately, it will be observed that the data indicate much higher
cation-absorption rates from solutions at pH 7.0 than from solutions at pH 4.0,
the rates at pH 7.0 being more than three times as high as the corresponding
rates at pH 4.0.   This again emphasizes the point brought out by Clark and

Shive (1) that the pH of the culture medium appears to be the dominating factor in determining the rate of absorption of cation nitrogen by the tomato plant. On the other hand, the rate of absorption of anion nitrogen from solution at pH 4.0 was somewhat higher than the corresponding rate of absorption from solution at pH 7.0, but pH of the culture solution did not have the same pronounced influence in determining the rates of anion-nitrogen absorption as it did in determining the rates of cation-nitrogen absorption. This also is in entire agreement with the results obtained by Clark and Shive (1) with tomato plants at later stages in the growth cycle.

The point to be emphasized in these studies with young tomato seedlings is that the average maximum ammonium-nitrogen absorption rate, at pH 7, although decidedly higher than the corresponding rate at pH 4, was still significantly lower than the minimum-nitrate absorption rate at pH 7. In this

TABLE 2

Milligrams of ammonium and nitrate nitrogen, per 10 gm. green tissue, absorbed in 6 hours by 10-day-old tomato plants from culture solutions at two pH levels

CULTURE NUMBER	pH	NUMBER OF PLANTS	GREEN WEIGHT	NITROGEN ABSORBED			
				NH₄-N		NO₃-N	
				Individual	Average	Individual	Average
			gm.	mgm.	mgm.	mgm.	mgm.
1	4.0	238	13.7	0.67	0.85	4.50	3.95
2	4.0	213	13.7	1.22		3.62	
3	4.0	234	13.7	0.90		4.34	
4	4.0	215	15.2	0.61		3.34	
5	7.0	228	12.2	2.35	2.64	3.30	3.19
6	7.0	234	10.0	2.87		3.47	
7	7.0	230	10.8	2.45		3.72	
8	7.0	231	11.5	2.88		2.25	

respect the tomato shows a very distinct difference from the other species previously studied. It will be recalled from the work of Stahl and Shive (6, 7) and from that of Naftel (4), that in the case of oats, buckwheat, and cotton the ammonium-nitrogen absorption predominated over the nitrate-nitrogen absorption in the young stages of growth and then rapidly decreased, and that the nitrate-nitrogen absorption rates gradually increased and assumed the ascendancy during the later stages of the growth cycle.

In the tomato plant, on the other hand, under optimum conditions, nitrate-nitrogen absorption rates generally predominated over ammonium-absorption rates throughout the entire growth cycle. In this respect the facts indicate that the tomato plant may be regarded distinctly as a nitrate absorber when nitrogen is present in the growth medium in both the cation and anion forms simultaneously and in approximately equal molar proportions, but at no time

in the active growth cycle has it been found that the tomato plant actually ceases to absorb cation nitrogen in the presence of an available supply of this form.

Like other species previously studied, the tomato plant showed maximum absorption rates of cation nitrogen during the seedling phase of growth. These rates then rapidly declined with the age of the plant and seldom predominated over the anion-absorption rates. Unlike other species previously studied, the tomato plant also showed maximum absorption rates of anion nitrogen during the seedling phase of growth, and these rates exhibited marked superiority over maximum rates of absorption of cation nitrogen.

Because of the pronounced tendency of the tomato plant to remain very vegetative as long as favorable growth conditions prevail, it is to be expected that this plant should exhibit some absorption characteristics different from those of other species previously studied. However, this does not explain why some species are predominantly nitrate absorbers, while other species are predominantly ammonium absorbers, when the two forms of nitrogen are simultaneously present in the growth medium.

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APPARATUS FOR THE DETERMINATION OF CO_2 IN CULTURE SOLUTIONS[1]

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During an investigation of the relationship between the aeration of culture solutions and the rates of nitrogen absorption by the plants growing therein, it became necessary to make quantitative determinations of the soluble carbon dioxide content of the culture solutions at periodic intervals. In order to make these determinations without exposing the test samples to the atmosphere, which might interfere with the accuracy of the tests, the special apparatus herein described was designed and used.

The acidimetric method of analysis was used, a description of which may be found in any standard work on quantitative analysis (3). Briefly stated, this method involves the absorption of the carbon dioxide by standard alkali, through which it is bubbled, and the precipitation as the insoluble barium salt of the carbonate thus formed. The exact amount of alkali thus neutralized is calculated by titrating, with standard acid, the excess alkali remaining after absorption is complete. The amount of carbon dioxide present in the solution may then be calculated directly in terms of parts per million, or milligrams of CO_2 per liter of solution.

Mack (2) describes an ingenious apparatus embodying the aforementioned principle, which he used to measure the amount of CO_2 evolved from solution cultures. It was so devised as to enable one to titrate the absorbing alkali without exposing it to the atmosphere, precluding what might have been a serious error. The absorption tower in this device consisted of a large-bore glass tube partially filled with small glass beads, a unit which is frequently used in apparatus of this type.

In the present work of estimating rather minute quantities of CO_2, it was observed that in order thoroughly to remove the residual alkali from the absorption tower to the titration flask, a very appreciable quantity of wash water was necessary. As a consequence, the original alkali, which was necessarily quite dilute because of the small quantities of CO_2 to be assayed, was just so much further diluted, and this interfered with the obtaining of a sharp end point in the titration.

The apparatus herein described circumvents this difficulty. Since the

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investigation was concerned with the total dissolved CO_2 in a solution at a specified time, the method devised by Allison and Shive for collecting the sample and aliquoting it with a minimum exposure to the atmosphere was here used (1).

The apparatus as diagrammed in figure 1, is made up of two distinct sections. The section on the left, containing the long tube A, served for the absorption of the carbon dioxide by the alkali and the subsequent titration. That part of the apparatus on the right, including tube B, served for the liberation of the CO_2 from the sample solution. This was accomplished by making the solution strongly acid with approximately 2 N HCl.

In order to describe the apparatus with as little confusion as possible, the various steps in the analysis of a sample will be followed through in order.

The first step is to remove the absorption tube A and the reaction tube B from the apparatus after the previous determination. These are thoroughly cleaned and rinsed, as are all of the parts contained within the tubes. Tube A is replaced, after a few drops of phenolphthalein indicator have been introduced into it. Tube B, now containing 5 cc. of 2 N HCl which later provides the means of acidifying the test solution sample when it is admitted to tube B, is also replaced. The sample bottle containing the test sample is next attached as shown in the figure.

Suction is then applied at Y, with the pinchcock P_3 closed, the stopcock of burette F closed, and the two-way stopcock K above the sample bottle turned as shown in the figure. Air enters the system through the soda-lime tube M, and all parts of the system between M and Y are thus freed of carbon dioxide.

Suction is then stopped at Y and applied at X with the same water pump. With pinchcock P_1 open, stopcocks of all burettes, C, E, and F closed, and P_4 on tube D closed, air enters this part of the system through the soda-lime tube L until it, too, is filled with CO_2-free air. This is demonstrated by closing the pinchcock P_1 and allowing the air stream to pass through the telltale tube T_1, filled with saturated barium hydroxide solution. Any trace of carbon dioxide in the air stream can be at once detected by this means. After it is certain that all parts of the apparatus are free of carbon dioxide, it is then ready for the analysis of the test sample.

Continuing suction at X, the standard alkali is admitted to the 100-cc. thick-walled test tube A. The alkali is accurately measured from burette E and conducted into tube A by the small funnel O on the end of a long glass tube of from 1.5-to 2-mm. bore, as shown in the diagram. Through this long inlet tube are also drawn the air and, later, the air-CO_2 mixture, from the reaction tube B, as well as all other material which entered the absorption tube A at any time during the determination. This, as well as the manner of admitting the sample solution to the reaction tube B, makes it possible to carry through the entire process without opening any part of the apparatus to the atmosphere.

The long inlet tube just mentioned is provided with a small hooked bend at its tip, on which rests a special piece of spirally coiled glass tubing. This glass

coil is formed around a central hollow glass tube of 1.3 cm. diameter as a core, and is sealed at both ends to prevent entrance of any materials. The glass coil fits rather loosely against the walls of the absorption tube, but just tightly enough so that the bubbles of air, or air-CO_2 mixture, released at the bottom
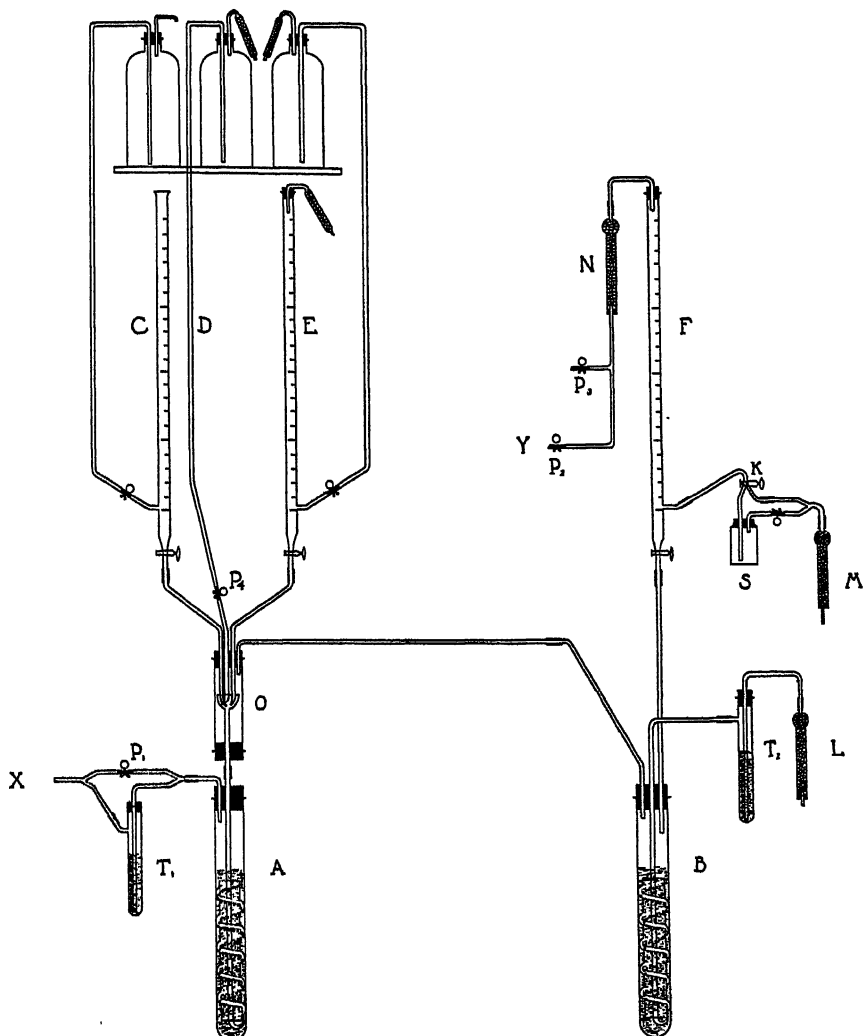
FIG. 1. APPARATUS FOR THE DETERMINATION OF CO_2 IN CULTURE SOLUTION

do not rise vertically through the alkali in the absorption tube A, but instead slowly follow a spiral path, guided by the surface of the glass coil between the walls of tube A and the core of the spiral. A sufficient quantity of alkali is used to cover completely the coil in the tube. By means of a screw clamp at

X the suction can be carefully regulated so that the bubbles are allowed to pass rather slowly through the alkali, thus insuring complete absorption of the CO_2 as it is released from the solution sample in tube B and carried over into tube A.

The next step in the procedure is to open the two-way stopcock above the sample bottle S in the opposite direction, and to draw some of the sample solution up into burette F. This is easily accomplished by applying suction at P_3. The level of the liquid in the burette is easily regulated by the two-way stopcock, allowing any excess solution to flow back into the bottle. Inasmuch as the test solution is now in contact with CO_2-free air in the burette, it is important that the next step in this procedure should be performed as quickly as possible, in order to prevent any appreciable diffusion of the gas from the solution into the air. Consequently, a measured quantity of the sample solution is quickly drawn from the burette into the reaction tube B, the suction at X being continued all the while.

Inasmuch as the acid is already in the reaction tube B, the sample solution becomes acidified as it is admitted, and thus liberation of carbon dioxide starts immediately. The reaction tube B contains a glass spiral and core similar to that in the absorption tube A. As the air stream entering through the soda-lime tube L passes in small bubbles spirally upward through the acidified sample solution, the released carbon dioxide is carried along with it, and thence passed over into the absorption tube A.

This aerating process is continued during a period of 10 minutes for each test. It had been previously ascertained that all the carbon dioxide can be removed in this period of time from a solution sample such as was here used. At the end of this 10-minute interval, the excess alkali not neutralized by the carbon dioxide is titrated by admitting standard acid directly from the burette C into the small funnel O, without opening any part of the system. During the titration, suction is continued in order to draw the acid down into the absorption tube A, and the bubbles continually passing through the solution serve effectively to mix the acid and the alkali. When the end point of the titration is approached, CO_2-free distilled water is admitted through the tube D to rinse the walls of the funnel and inlet tube. The reading of the burette C gives the exact quantity of standard acid required to neutralize the excess alkali, and by difference the exact quantity of carbon dioxide liberated from the sample aliquot is then computed in terms of parts per million.

Approximately 0.02 N NaOH was used as the absorbing agent in these determinations, and this was standardized against 0.07 N H_2SO_4, methyl red being used as indicator. For the titration, 0.1 N HCl was employed, with phenolphthalein as indicator. This acid was made up with barium chloride in solution at a 1 per cent concentration. This solution contained a sufficient quantity of barium chloride per cubic centimeter (calculated) to react in excess with all the carbon dioxide released from any of the samples analyzed.

Tables 1 and 2 illustrate, in a measure, the degree of accuracy which may be attained with this apparatus. Table 1 gives the values for CO_2 calculated and

recovered from samples of a CO_2-free culture solution to which had been added known quantities of Na_2CO_3.

Table 2 presents the quantitative data representing the CO_2 obtained by the analysis of duplicate samples of culture solutions which had previously been in contact with the roots of experimental plants for a definite period of time. The samples were taken while the roots of the plants were still immersed in the solutions maintained at different pH levels. The table is here introduced to

TABLE 1

Analysis of known samples of a culture solution

SAMPLE	CO₂	
	Calculated	Recovered
	mgm.	*mgm.*
10 mgm. Na₂CO₃ in each sample of the culture solution { 1.........	4.09	4.00
2.........	4.09	4.15
3.........	4.09	4.03
4.........	4.09	4.12

TABLE 2

Analysis of duplicate unknown samples of culture solutions after contact with the roots of growing plants during a definite time interval

SAMPLE	pH OF CULTURE SOLUTIONS	CO₂
		mgm.
1	4	0.61
Duplicate	4	0.63
2	7	1.32
Duplicate	7	1.39
3	4	0.72
Duplicate	4	0.72
4	7	2.38
Duplicate	7	2.42

indicate the approximate agreement which may be expected between tests of duplicate samples when the analyses are carried out with the apparatus here described.

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THE RELATION OF POTENTIAL ALKALINITY TO THE AVAILABILITY OF PHOSPHATE IN CALCAREOUS SOILS

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The use of phosphate fertilizers in the irrigated regions of the Southwest has increased notably during the last few years and is a practice which is yielding profitable returns. The soils are with few exceptions alkaline calcareous types, and the problems connected with phosphate fertilization are extremely varied and of great economic as well as scientific interest. There is no deficiency of potential phosphate reserve in any of these soils. The apparent deficiency is due to characteristic soil properties which govern the solubility and availability of the phosphate and the absorptive activities of the roots. In fact, even a supply of phosphate in the soil solution does not necessarily insure the crop against phosphate starvation.

PHOSPHATE COMPOUNDS AND THEIR SOLUBILITY

Phosphate is present in these alkaline calcareous soils, in most part, as carbonato-apatite (calcium carbonato-phosphate), a compound composed of three mols tri-calcium phosphate and one mol calcium carbonate, with small amounts of iron and aluminum phosphate (4). Extensive solubility studies on these soils have shown that those within the reaction range represented by the limits pH 8.0 and 8.5 contain the smallest amounts of soluble phosphate (3). The reason for this is illustrated by the curves given in figure 1, in which solutions of ferric phosphate, aluminum phosphate, dicalcium phosphate, and phosphate rock were adjusted to various concentrations of hydrogen or hydroxyl ions and the changes in solubility noted. The solubility curves for the calcium phosphates are of the descending order as pH is increased, whereas the solubility curves for iron and aluminum phosphates are of an ascending order with increase in pH where black alkali (NaOH) is present and of the descending order where lime alkalinity [Ca(OH)$_2$] is present. Under many soil conditions carbonato-apatite is a readily available form of phosphate. Reference is made to acid soils, neutral soils, and certain slightly alkaline soils which contain no solid phase calcium carbonate. Under the soil conditions which exist in the Southwest, namely, an alkaline reaction and an abundance of solid phase calcium carbonate, its solubility is at a minimum and usually fails to supply the requirements of the crop.
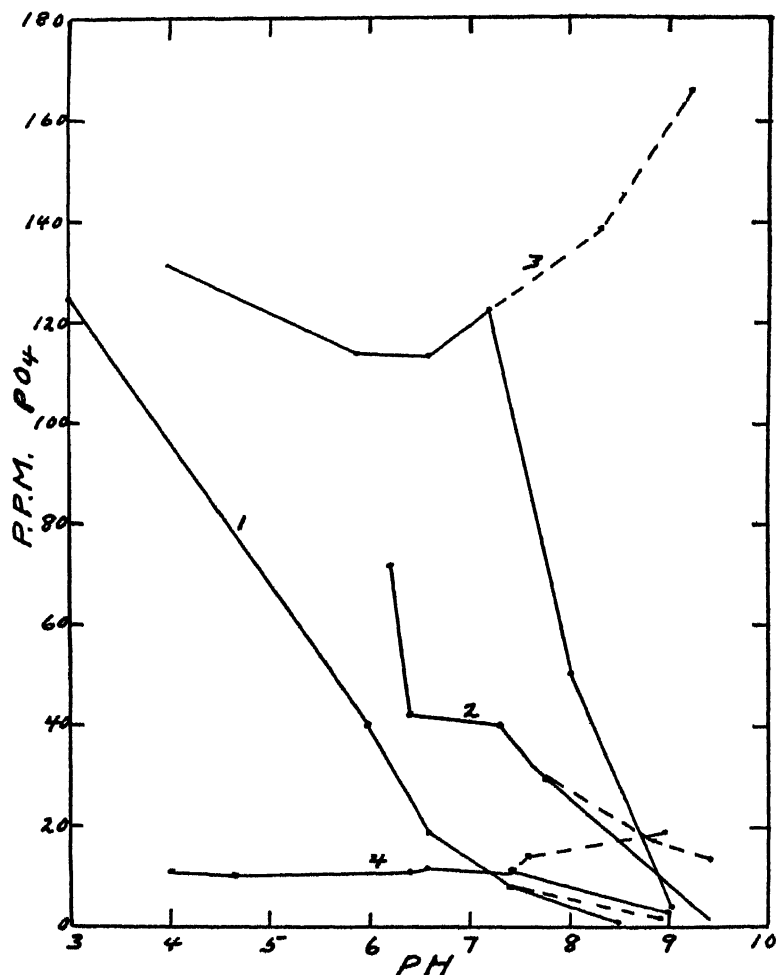
FIG. 1. DESCENDING ORDER OF SOLUBILITY OF CALCIUM PHOSPHATES AND ASCENDING
ORDER OF SOLUBILITY OF PHOSPHATES OF IRON AND ALUMINUM WITH
INCREASE IN pH

1. Phosphate rock; 2. Di-calcium phosphate; 3. Aluminum phosphate; 4. Iron phosphate.
Broken line represents sodium hydroxide alkalinity; and solid line, calcium hydroxide alkalinity.

FACTORS OTHER THAN SOLUBILITY WHICH AFFECT PHOSPHATE AVAILABILITY IN ALKALINE-CALCAREOUS SOILS

Before the study of phosphate availability in southwestern soils had progressed very far it became evident that solubility relations were not the only important factors involved in the apparent phosphate deficiency. The ionization of ortho-phosphate at alkaline reactions (2) and the effect of OH ions on

the absorption of phosphate ions by plants (1) were found to be intimately associated with this phosphate deficiency problem. To review this work briefly, ortho-phosphates undergo step ionization as follows:

$$H_3PO_4 \rightarrow H_2PO_4^- \rightarrow HPO_4^{--} \rightarrow PO_4^{---}$$
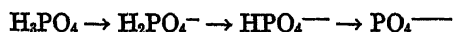
Plants were shown to possess a very definite preference for the H_2PO_4 ion, and, since this ion exists largely under acid soil conditions and is present in very small proportion in alkaline soils, it is self-evident that a greater concentration of soluble phosphate in the soil solution of alkaline soils will be required to supply the plants' requirement for H_2PO_4 ion. On growing plants in water cultures it was found that absorption of phosphate ions did not take place at reactions above approximately pH 7.6. This means that crops cannot absorb phosphate ions when the pH of the root-soil contact film is higher than pH 7.6. Thus phosphate nutrition in alkaline calcareous soils is not only a problem in solubility but also one in which soil environmental conditions, which interfere with normal absorptive processes of plant roots and with the proper ionization of ortho-phosphate, must be corrected. In other words, the pH of the soil solution or of the root-soil contact film must be reduced.

ALKALINITY OF THE SOIL SOLUTION IN QUANTITATIVE TERMS

Many of the observations made during soil studies in this laboratory have led to the conclusion that, except where present in unusually excessive concentrations, black alkali is not toxic in most so-called black alkali soils, but rather that such soils are infertile because of the reduced absorption of ions which obtain in the presence of hydroxyl ions (1). This is borne out by the data given graphically in figure 2, in which the concentrations of sodium carbonate, sodium hydroxide, and hydroxyl ion at various pH values between 7 and 10 have been calculated from ionization constants. The concentration of OH ion even at pH 10.0 is much less than we have been generally led to believe and, therefore, the reduction of this to pH 7.6 does not appear to be a difficult task. Although the problem is not so simple as the graph indicates, there are feasible methods of accomplishing this end.

Since all plants are fitted by nature to correct the undesirable environment created by the presence of hydroxyl ions in the soil solution, they enjoy a varied but limited immunity toward black alkali. Reference is made to the exudation of carbon dioxide by living roots. In many soils, roots are aided by the respiration of the soil microflora, but in semi-arid soils there is so little organic matter that crops get very little assistance from this quarter. All of our investigations point to carbon dioxide as the greatest growth-limiting factor in semi-arid soils and, in fact, the very key to their productivity. It is self-evident that free carbon dioxide cannot exist in the presence of hydroxyl ions. If is further self-evident that carbon dioxide is the only means by which the crop can reduce soil alkalinity and thereby promote normal absorption of phosphate.

Figure 2 shows that it should be a relatively simple task for plant roots to reduce the pH of the soil solution when this is not greater than pH 8.5, but that it becomes increasingly difficult above pH 8.5 because of the rapidly ascending nature of the curve above this point. It also is made increasingly difficult because of the fact that all soil solutions are buffered—some very strongly—and, too, in southwestern soils we cannot overlook the resistance to pH reduction which solid-phase calcium carbonate offers. These several fac-
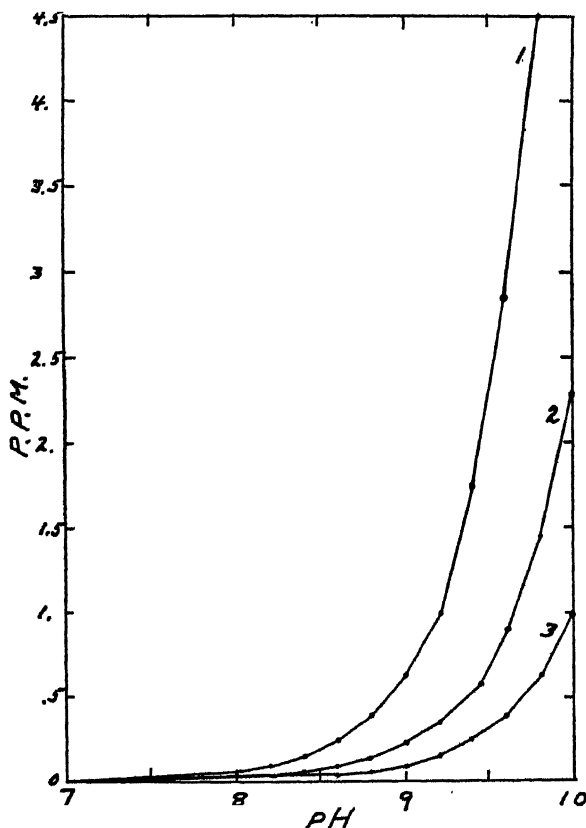
FIG. 2. RELATION BETWEEN pH AND PARTS PER MILLION OF: 1. SODIUM CARBONATE; 2. SODIUM HYDROXIDE; 3. HYDROXYL ION

tors demand such a great expenditure of energy by the crop in reducing or attempting to reduce the pH of the soil solution that the vitality of the crop suffers to such an extent that it becomes necessary to furnish aid artificially. Thus, in the Southwest, we must devise methods of supplying growing crop plants with a constant and adequate supply of carbon dioxide.

In view of the foregoing, it is not surprising to note that practically every procedure employed in the reclamation of alkaline soils is in reality an attempt

to supply the soil with carbon dioxide. Manure and other forms of organic matter supply potential carbon dioxide and stimulate the activities of the soil microflora; gypsum opens up the soil to better aeration; sulfur is oxidized to sulfuric acid, which in turn generates carbon dioxide from the calcium carbonate; and, finally, alkali-resistant crops are used largely because of the carbon dioxide which their roots exude. Thus, methods already in vogue in the cultivation of alkali soil types should be useful in improving phosphate availability. In this connection the remarkable improvement in phosphate availability obtained by Pittman (5) from manuring Utah soils is of interest.

POTENTIAL ALKALINITY OF IRRIGATION WATER

Although the foregoing methods of improving the fertility of alkali soils lead toward greater phosphate availability, there is one phase of the fertility problem which has not received adequate attention and one which should have an important bearing on the phosphate deficiency problem. Reference is made to the *quality* of irrigation water as related to plant-foot absorption. The term *quality* does not refer here to salt concentration, which is the general use of the term, but rather to the potential alkalinity both of the water itself and of the water after contact with the soil in the field. The reaction of alkaline soils is almost entirely a function of the degree of hydrolysis of the sodium-clay exchange complex, which in turn is a function of the composition of the irrigation water and the soil solution. Most irrigation waters are slightly alkaline, and it is not uncommon to find some showing phenolphthalein alkalinity. Furthermore, the reaction of irrigation water is subject to change: for example, bicarbonates change to carbonates on evaporation under the extreme temperatures of the semi-arid climate, alga and other aquatic plants increase the pH of water by extracting their carbon dioxide from the soluble bicarbonates in the water, and finally the hydrolysis of the sodium-clay compound is subject to fluctuation, upon dilution, at each irrigation. All three of these conditions have a definite bearing on phosphate solubility and its subsequent absorption by crops and not only warrant serious consideration in the general problem of phosphate nutrition in alkaline calcareous soils, but offer a medium for solution of the problem; that is, the solution of the problem appears to lie in an economic method of reducing the pH of alkaline irrigation waters. Such a procedure should not only increase phosphate availability but should also tend to increase the rate of water penetration and correct many other factors which are involved in the low fertility of alkaline soils.

EXPERIMENTAL

In support of the preceding discussion, the following experiments, illustrative of the investigations which have been conducted on the problem, are presented.

Experiment 1

The first experiment involves the changes in pH which take place when soils come in contact with water. The three soils selected are representative of the irrigated soils of Arizona. The following is a brief description of the soils, all of which are calcareous and respond to phosphate fertilization:

1. A black alkali soil, sandy loam alluvial type which also contains large amounts of white alkali.
2. A clay-loam soil, which tends to puddle and in which water penetration is poor.
3. A sandy loam soil which has good penetration.
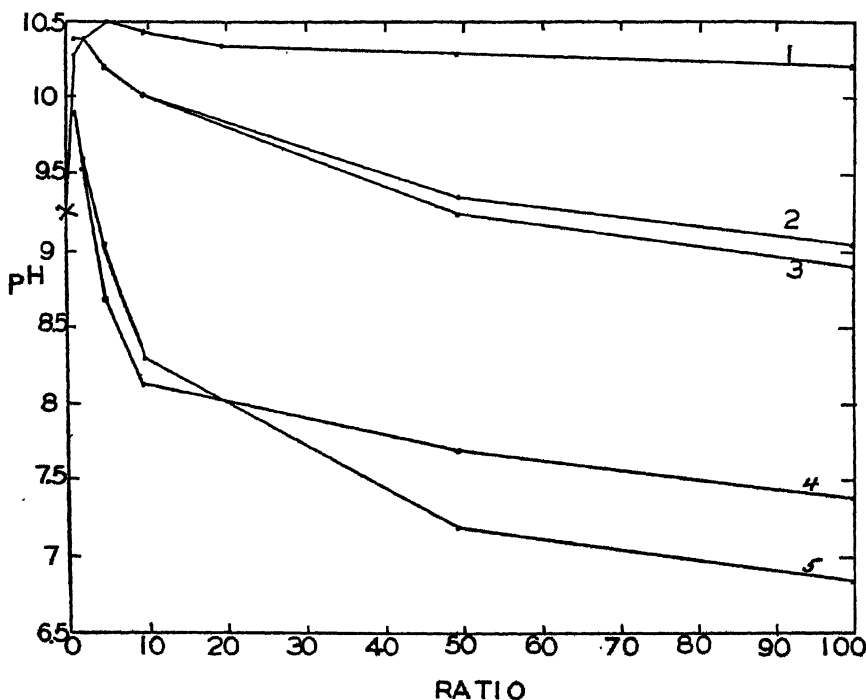
Fig. 3. Effect of Varying the Soil-Water Ratio on pH of Soil Number 1

1. Boiled distilled water; 2. Boiled tap water; 3. Unboiled tap water; 4. Distilled water plus CO_2; and 5. Tap water plus CO_2.

The soil solution was obtained by packing 1,000 gm. of soil of optimum moisture content in glass percolators, the tips of which dipped below the surface of neutral mineral oil contained in large test tubes, thus preventing contact of the soil solution with air as it dropped from the percolators. Alcohol was used as a displacing liquid. Only 100 cc. of each of the soil solutions was collected, as this was sufficient for the tests. The pH values of these soil solutions were determined with the hydrogen electrode and checked with indicators. The values obtained (pH 9.25 for soil 1, 8.0 for soil 2, and 8.25 for soil 3) are represented in figures 3, 4, and 5 by crosses (X).

To illustrate the effects of dilution on the pH of these soils, that is, the influence of the soil-water ratio upon pH, soil suspensions were prepared in which the ratio of soil to water varied from 1:1 to 1:100. In order to show the manner in which water brings out the potential alkalinity, several different kinds of water were used; namely, distilled water which had been boiled to remove all carbon dioxide, tap water which had been boiled, tap water unboiled, distilled water containing 105 p.p.m. carbon dioxide, and tap water containing 230 p.p.m. carbon dioxide. Boiled distilled water was used to bring out the
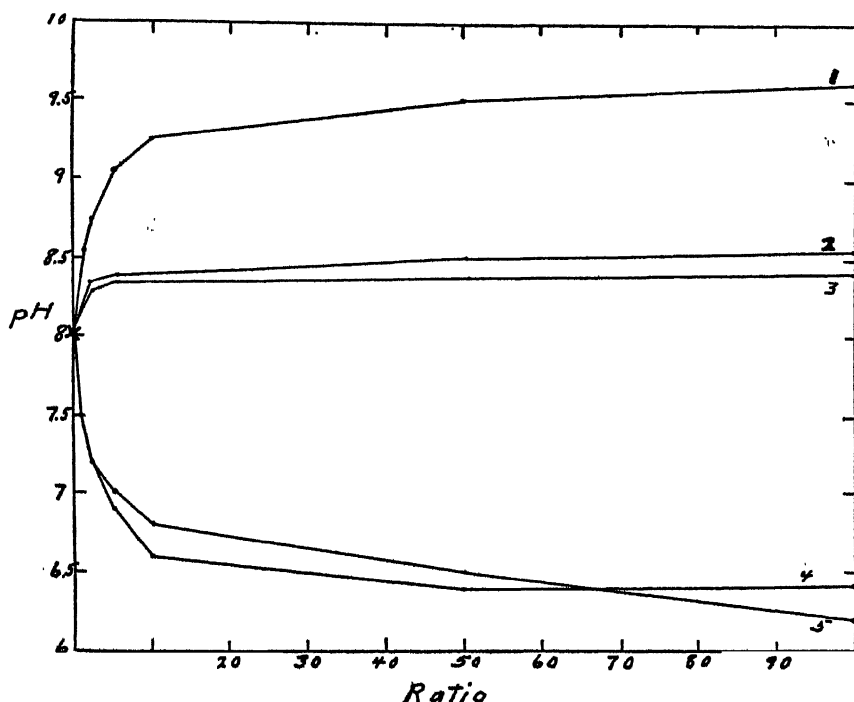
FIG. 4. EFFECT OF VARYING THE SOIL-WATER RATIO ON pH OF SOIL NUMBER 2
1, 2, 3, 4, and 5, same as in figure 3

maximum potential alkalinity; unboiled tap water, to represent irrigation water (well buffered); boiled tap water, to represent the conditions arising when irrigation water is allowed to evaporate in reservoirs or in the field where penetration is slow; and the two waters containing carbon dioxide were used to illustrate the conditions which arise where acidified irrigation water is run on a field of calcareous soil. The pH values were determined with the hydrogen electrode in all cases except in soils 2 and 3 where water containing carbon dioxide was used. In each of these latter soils the pH was determined with indicators. The data obtained are presented graphically in figures 3, 4, and 5.

Soil 1.—This is a black alkali soil, the soil solution of which contains 226,000

p.p.m. salts (total solids) and has a pH of 9.25. At low soil-water ratios
there is a rapid increase in pH of the soil for both distilled water and tap
water, reaching a maximum of pH 10.5 for the distilled water at a 1 to 5 ratio
and tapering off to pH 10.2 at a 1 to 100 ratio because dilution of sodium car-
bonate and hydroxyl ion is greater than the increase in hydrolysis of sodium
clay with dilution. Where a buffered water is used (tap water) the maximum
pH is at 1 to 2 soil-water ratio, from which the pH tapers off very rapidly to
pH 9.1 for the boiled tap water and to 8.85 for the unboiled tap water. When
the waters contain carbon dioxide there is a rapid decrease in pH with increase
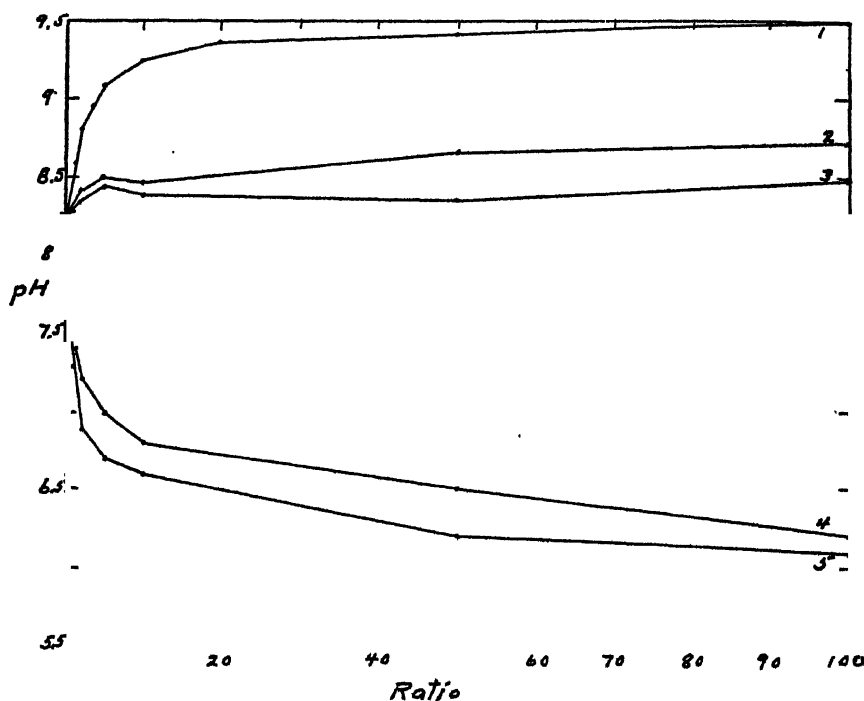
FIG. 5. EFFECT OF VARYING THE SOIL-WATER RATIO ON pH OF SOIL NUMBER 3
1, 2, 3, 4, and 5, same as in figure 3

in soil-water ratio, and here again the buffered water offers the greater resist-
ance to reduction in pH, as it did to increase in pH in absence of carbon
dioxide.

Soil 2 and 3.—The general character of the curves for these two soils is the
same as for soil 1, except that reduction of pH by the waters containing carbon
dioxide is more completely within the feeding range.

It is recognized that increase in pH with increase in soil-water ratio is not
so great for tap water as for distilled water, but it must be remembered that
reduction in pH of the tap water-soil mixture requires more energy because of

the more highly buffered nature of the system. There seems no question but that there will be an increase in the pH of the soil solution or soil-water system with each irrigation, depending upon the potential alkalinity of the water and the amount of sodium clay in the soil. This pH will, of course, be ultimately reduced to the actual pH of the soil solution as the percentage of water in the soil is reduced. It is thus apparent that the frequent irrigation of semi-arid alkaline soils is not conducive to maximum phosphate availability and absorption by crops, and the whole suggests the importance of a study of the pH of irrigation waters if any great advance in phosphate availability in these soils is to be effected.

Experiment 2

It is plainly evident from experiment 1 that some means of generating carbon dioxide in the soil will help greatly to solve the phosphate problem. The most economical means of accomplishing this in Arizona is by the use of sulfuric acid in small amounts in the irrigation water. A 0.001 N solution of

TABLE 1

Phosphate (PO_4) in dry corn plants irrigated with water of pH 3.0, 7.0, and 8.5

REACTION OF WATER	PO₄ IN DRY CORN PLANTS GROWN ON		
	Soil 1	Soil 2	Soil 3
	per cent	*per cent*	*per cent*
pH 3.0	1.50	1.90	2.25
pH 7.0	1.46	1.51	1.55
pH 8.5	1.35	1.48	1.40

sulfuric acid contains 49 p.p.m. H_2SO_4 and gives a hydrogen-ion concentration of pH 3.0. Such a pH should be sufficiently low to generate the small amount of carbon dioxide required to reduce the pH of the soil solution and thus aid the roots in absorbing phosphate. With these facts in mind, the following experiment was conducted.

Three soils, thoroughly representative of our alkaline calcareous types, were selected. Three pots of each were prepared and planted to four corn plants per pot. One pot of each soil was irrigated with tap water adjusted to pH 3.0; another, with tap water adjusted to pH 7.0; and the third, with tap water adjusted to pH 8.5. After 6 weeks the plants were harvested, tops only, and their phosphate contents determined by chemical analysis. The results obtained are given in table 1. Each pot was fertilized with calcium nitrate and ammonium phosphate to insure the presence of ample available phosphate in the soil and thereby eliminate all factors except reaction and carbon dioxide.

This experiment conclusively demonstrates that the absorption of phosphate is governed by the pH of the irrigation water, that the use of alkaline waters reduces the absorption of phosphate by plants growing upon alkaline calcareous

soils, and that reduction of the pH with sulfuric acid, or the carbonic acid generated thereby, increases the absorption of phosphate. Field experiments indicate that similar results are obtainable in the field and are entirely economical with 60° Baumé sulfuric acid available in Arizona at $8 per ton F.O.B. the smelters.

<div align="center">CONCLUSIONS</div>

The phosphate problem on alkaline calcareous soils is not only one of low solubility of phosphate compounds present in the soils themselves, but also one in which the highly alkaline reaction of the soils interferes with the absorption by crops of added soluble phosphates.

The alkalinity of irrigation waters, both of themselves and in association with the potentially alkaline zeolitic compounds in the soil, makes for further reduced phosphate absorption by crops.

Reducing the pH of the irrigation water by addition of small amounts of sulfuric acid greatly increases the absorption of phosphate by plants irrigated with such water.

It is not unreasonable to assume that the addition of acid to irrigation waters will become a standard practice in the not distant future on soils of these types.

<div align="center">REFERENCES</div>

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SOME CHEMICAL AND PHYSICAL PROPERTIES OF NORMAL AND SOLONETZ SOILS AND THEIR RELATION TO EROSION[1]

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Soils locally designated as "alkali spots" or "slick spots" are found rather frequently in central Oklahoma bordering the Permian-Pennsylvanian contact. The Sandstone Hill region or Central Cross Timber soils area represents the outcrop of the Pennsylvanian period occurring farthest west in the state. This sandy area without sharp boundary lines separates the Eastern Prairies or Prairie Plains, an outcrop of the Pennsylvanian period, from the Central Prairies or Redbeds of Permian age. It is in or near the borders of these latter two provinces that the alkali spots are most numerous, though their occurrence is not infrequent throughout much of the section.

The presence of these so-called "alkali spots" is probably due to the accumulation of sodium salts in the sediments laid down by the receding sea, as the water in the deeper surface reservoirs evaporated as a result of arid conditions. The lateral extent of an individual alkali spot is usually quite distinct, indicating definite abrupt surface conditions at the time of deposition, accumulation, and formation of the solonchak soil.

As the soluble sodium salts reacted with the base exchange complex Na-clay formed. Rain water later removed the soluble salts to a greater or lesser extent and carried them down into the lower horizons of the profile; but the Na-clay formed, being insoluble in water, was not readily removed in this manner and tended to persist. As the soluble sodium salts were leached out of the surface soil by the rain water, hydrolysis of the Na-clay occurred and NaOH, its soluble product, caused the soil to become more or less alkaline in reaction and highly deflocculated. This retarded further leaching, and with this retardation and existing climatic conditions morphological features characteristic of solonetz soils developed.

The normal profile developed in the absence of salt accumulations has an A horizon varying in depth from just a few inches to 18 inches or more. It is of variable texture but in structure it is generally friable. The B horizon usually

is a sandy clay to stiff clay with a blocky, cubical, or angular cloddy structure. With the sandier types prismatic structures are often developed. Claypans have developed in some instances. The parent materials of the area are sandstone and shale.

The solonetz profile where erosion has not been severe usually presents four or five rather distinct horizons. The A_1 horizon is sandy loam in nature and usually ranges from 2 to 6 inches in depth. Its color is usually buff to light brown. Where drainage has been adequate, the A_2 horizon is usually darker brown than the surface layer, as a result of the accumulation of organic matter in this zone. It is not so sandy as A_1 but is usually of a loamy nature. The A_3 horizon is a very thin gray layer occurring over the caps of the columnar B_1 horizon. In some instances its appearance is not pronounced. The caps of the columns in contact with the A_3 horizon have a tendency to be rounded, although this development is not always pronounced. The B_1 horizon is variable in thickness but merges into a cloddy B_2 horizon and then into a more or less cemented structureless mass of high soluble-salt content. Limestone concretions occur frequently throughout the B horizon in many cases. Below this structureless horizon, which is of variable thickness, sandstone is usually encountered.

METHODS USED

The water-soluble constituents were determined by using 1:5 extractions with distilled water. The replaceable bases were determined by using neutral normal ammonium acetate as the replacing reagents. The term "active" is used to designate the amount of calcium or sodium soluble in and displaced by the ammonium acetate solution. The acetate leachate was treated as recommended by Salgado (2) and the sodium was determined by the Barber and Kolthoff (1) method.

The dispersion coefficient was determined by shaking, at the rate of 50 r.p.m., 4 gm. of air-dried soil with 400 cc. of distilled water in straight-walled bottles 12 cm. deep. The bottles were removed from the machine at the end of 24 hours and placed in an upright position for 24 hours, at the end of which time the top 5 cm. were carefully siphoned off and the number of centigrams of material in suspension in 100 cc. was recorded as the dispersion coefficient.

The mechanical analysis of the soils was determined by the Bouyoucos method.

EXPERIMENTAL DATA

The data presented were obtained largely from the exposed surfaces of the normal and the eroded solonetz profiles. Erosion had taken place at least to some extent on the normal soil. Tables 1 and 2 record the results of paired exposures of the normal profile and the nearby solonetz profile. The amounts of the various bases are expressed in terms of milliequivalents per 100 gm. of

TABLE 1

The dispersion coefficient and sodium-calcium ratios of the exposed B horizon of the solonetz profile compared with the A horizon of the normal profile

LOCATION IN STATE (NEAREST CITY)	EXPOSED A HORIZON OF NORMAL SOIL					EXPOSED B HORIZON OF SOLONETZ SOIL				
	Dispersion coefficient	Water-soluble Na	Active Na	Active Ca	Ca/Na ratio	Dispersion coefficient	Water-soluble Na	Active Na	Active Ca	Ca/Na ratio
		m.e.	m.e.	m.e.			m.e.	m.e.	m.e.	
Guthrie.........	1.22	0.11	0.86	1.75	2.03	8.14	1.64	6.44	1.65	0.26
Moore..........	0.72	0.06	0.22	2.85	12.95	18.30	9.60	11.87	3.30	0.28
Moore..........	0.42	0.13	0.25	1.83	7.32	2.64	1.10	3.03	2.52	0.83
Edmond.........	1.42	0.06	0.29	2.48	8.55	2.60	0.69	3.08	2.90	0.94
Oklahoma City...	1.16	0.07	0.27	2.65	9.81	4.20	2.52	4.59	3.20	0.70
Agra............	0.74	0.08	0.21	2.20	10.48	1.96	0.30	1.62	2.20	1.36
Shawnee.........	0.66	0.10	0.24	2.70	11.25	1.86	1.85	4.10	2.95	0.72
Meeker.........	0.90	0.04	0.05	1.75	35.00	3.58	4.08	6.55	1.45	0.22
Norman.........	0.62	....	0.14	2.48	17.72	2.94	1.89	5.94	4.95	0.83
Stillwater........	0.80	0.08	0.08	2.75	34.30	4.74	....	9.17	4.30	0.47
Claremore........	1.44	0.09	0.54	5.05	9.36	8.78	1.73	7.21	3.10	0.43
Claremore.......	1.14	0.24	0.56	3.60	6.43	2.72	1.33	3.86	2.65	0.69
Oilton..........	1.02	0.05	0.17	2.35	13.83	2.06	1.08	3.62	4.60	1.27
Nowata.........	0.66	0.09	0.46	2.20	4.77	1.98	0.91	4.19	2.20	0.52
Pawhuska.......	1.08	0.12	0.47	2.10	4.47	3.20	2.27	6.06	3.65	0.60
Claremore.......	0.94	0.05	0.44	2.45	5.57	1.80	0.82	3.00	2.90	0.97
Claremore.......	2.18	0.08	0.31	6.90	22.28	2.21	8.89	11.19	9.75	0.87
Valley..........	0.74	0.14	0.90	3.50	3.89	6.18	2.24	6.22	4.40	0.71
Lela............	1.58	0.04	0.51	1.70	3.33	9.50	....	7.66	1.90	0.25
Mannford.......	0.78	0.09	0.31	2.05	6.61	3.32	1.28	3.99	2.00	0.50
Chelsea..........	0.86	0.15	0.45	1.60	3.55	1.38	0.51	2.22	1.75	0.79
Hominy.........	1.30	0.06	0.52	2.85	5.47	2.80	1.38	4.74	1.60	0.34
Stillwater*.......	0.76	0.06	0.39	2.80	7.17	2.96	2.65	3.94	1.75	0.44
Stillwater.......	0.40	0.12	0.12	1.20	10.00	1.32	2.11	3.04	0.75	0.25
Stillwater.......	0.96	0.11	0.14	1.40	10.00	1.36	0.72	2.09	1.25	0.60
Stillwater.......	0.80	....	0.04	1.65	41.25	1.27	....	2.71	1.20	0.44
Stillwater.......	0.76	....	0.08	1.45	18.13	1.28	0.72	2.33	0.75	0.32
Stillwater.......	0.64	....	0.13	1.30	10.00	2.08	....	4.38	1.25	0.29
Stillwater.......	1.02	0.05	0.27	1.35	4.98	2.78	1.75	3.76	1.45	0.39
Stillwater.......	0.90	0.03	0.25	1.10	4.45	1.66	....	2.21	0.95	0.43
Stillwater.......	1.30	....	0.15	0.95	6.33	2.22	....	3.77	2.25	0.60
Glencoe.........	0.72	0.08	0.19	0.85	4.52	23.52	2.89	10.95	1.95	0.18
Average.......	0.96	0.08	0.31	2.31	11.12	4.29	2.19	4.99	2.61	0.58

* These soils are not paired samples, but were collected approximately one-fourth mile from each other.

soil. Table 3 shows the pH values of 19 paired soil samples before and after being leached with distilled water.

TABLE 2

The water-soluble and active base content of some typical solonetz profiles

LOCATION AND DEVELOPMENT	DEPTH	WATER-SOLUBLE			ACTIVE					
		Na	Ca	Ca/Na	Na	K	Ca	Mg	Di/Mono	Ca/Na
	inches	*m.e.*	*m.e.*		*m.e.*	*m.e.*	*m.e.*	*m.e.*		
Stillwater-Farm A:										
Normal profile, horizon A.....	0– 6	0.25	0.59	2.36	0.66	0.16	4.05	1.81	7.10	6.13
Solonetz, exposed horizon B....	0– 1	1.27	0.21	0.17	2.99	0.51	3.10	3.04	1.75	1.03
Solonetz, exposed horizon B....	1–12	1.20	0.27	0.23	5.07	0.51	4.45	3.83	1.48	0.88
Solonetz, exposed horizon B....	12–24	3.71	0.25	0.07	7.78	0.90	5.55	4.33	1.14	0.71
Solonetz, exposed horizon B....	24–36	0.70	0.54	0.77	4.02	1.44	4.05	3.58	1.48	1.01
Solonetz, exposed horizon B....	36–48	0.63	0.14	0.22	4.25	0.23	3.65	2.88	1.46	0.86
Stillwater-Farm B:										
Normal profile, horizon A.....	0– 6	0.38	2.91	7.66	1.05	0.77	6.90	3.21	5.55	6.56
Solonetz, exposed horizon B....	0– 1	7.95	3.02	0.38	12.62	0.72	4.80	3.58	0.68	0.38
Solonetz, exposed horizon B....	1–12	7.88	0.87	0.11	9.29	0.92	4.75	4.89	0.95	0.51
Solonetz, exposed horizon B....	12–24	7.97	7.55	0.95	11.38	0.37	8.75	4.69	1.15	0.77
Solonetz, exposed horizon B....	24–36	3.28	6.60	2.01	8.32	0.80	13.60	3.24	1.85	1.63
Stillwater-Farm C:										
Normal profile, horizon A......	0– 6	0.35	0.40	1.14	0.84	0.29	5.90	1.98	7.02	7.07
Solonetz, exposed horizon B....	0– 1	3.11	0.40	0.13	6.88	1.02	8.75	3.24	1.52	1.27
Solonetz, exposed horizon B....	1–12	3.26	0.40	0.12	5.78	....	11.25	3.62	....	1.95
Solonetz, exposed horizon B....	12–24	3.91	0.27	0.07	5.46	0.74	11.60	3.62	2.45	2.12
Solonetz, exposed horizon B....	24–36	2.03	0.40	0.20	7.64	0.79	7.45	3.24	1.27	0.98
Solonetz, exposed horizon B....	36–48	1.35	0.16	0.12	9.00	0.83	6.30	2.80	0.93	0.70
Stillwater-Farm D:										
Normal profile, horizon A......	0– 6	0.10	0.22	2.20	2.03	0.32	5.00	1.19	2.63	2.47
Solonetz, exposed horizon B....	0– 1	2.17	0.33	0.15	3.59	0.28	3.35	1.90	1.35	0.93
Solonetz, exposed horizon B....	1–12	3.58	0.65	0.18	7.33	0.81	6.90	3.66	1.30	0.94
Solonetz, exposed horizon B....	12–24	3.82	0.54	0.14	7.46	0.46	15.50	4.56	2.53	2.08
Solonetz, exposed horizon B....	24–36	1.85	0.57	0.31	3.97	0.60	8.90	3.13	2.63	2.24
Stillwater-Farm E:										
Normal profile, horizon A......	0– 6	0.45	0.22	0.49	1.29	0.32	5.70	3.01	5.43	4.43
Solonetz, exposed horizon B....	0– 1	0.83	0.25	0.30	3.39	0.49	4.20	3.21	1.91	1.24
Solonetz, exposed horizon B....	1–12	1.95	0.33	0.17	15.00	0.56	6.35	4.69	0.71	0.42
Solonetz, exposed horizon B....	12–24	3.84	0.27	0.07	6.99	0.79	7.70	5.14	1.65	1.10
Solonetz, exposed horizon B....	24–36	3.34	0.30	0.09	7.50	0.58	20.75	5.14	3.11	2.77
Solonetz, exposed horizon B....	36–48	3.95	1.08	0.27	5.08	0.79	9.70	4.20	2.37	1.91

Although some textural differences existed among the various soils, the general average indicates rather closely the textural conditions of the surface of

TABLE 3

The pH of 19 typical paired soil samples before and after leaching with distilled water

LOCATION	A HORIZON OF NORMAL SOIL PROFILE			B HORIZON OF SOLONETZ PROFILE		
	Soil	Leachate	Leached soil	Soil	Leachate	Leached soil
1	6.48	7.06	7.08	8.67	7.78	8.52
2	6.37	6.65	6.61	8.59	7.80	9.08
3	6.61	6.28	6.56	7.70	7.52	8.03
4	6.80	6.93	7.93	8.07	7.12	8.17
5	5.85	6.96	6.44	8.00	6.93	8.36
6	6.37	6.23	6.56	8.17	6.93	8.48
7	7.12	6.61	7.13	8.54	7.38	8.50
8	6.46	6.72	6.58	6.59	7.28	8.54
9	5.85	6.27	5.88	7.06	7.76	7.68
10	6.14	6.23	6.63	7.54	6.90	8.00
11	6.27	6.20	6.38	8.59	7.72	8.76
12	5.75	6.86	6.66	7.32	7.01	7.76
13	6.05	6.44	6.72	8.46	7.63	8.59
14	6.20	6.30	6.73	7.88	7.01	8.07
15	6.00	6.27	6.20	7.20	7.24	7.58
16	6.06	6.27	6.30	8.10	....	....
17	5.92	6.10	6.34	7.93	7.65	8.31
18	5.60	6.35	6.09	7.08	6.90	7.17
19	7.00	6.10	6.98	7.76	7.13	8.15

TABLE 4

The textural relationships of the soils studied

SOIL	HORIZON	SAND	SILT	CLAY	DISPERSION COEFFICIENT
		per cent	*per cent*	*per cent*	
Solonetz 1............................	A	45.60	30.00	24.40	0.18
	B	25.60	32.00	42.40	15.50
Solonetz 2............................	A	63.60	19.00	17.40	0.30
	B	55.60	17.00	27.40	17.54
Solonetz 3........	A	36.60	34.00	29.40	0.02
	B	53.60	10.00	36.40	22.28
Normal (samples average 32).............	A	43.77	26.68	29.55	
Solonetz (samples average 32)...........	B	38.38	22.88	38.74	

the normal soils. In table 4 are recorded the dispersion coefficients and mechanical analyses of the A and B horizons of three different solonetz soils indicating the variations in texture of these soils.

TABLE 5

The dispersion coefficient of three badly eroded solonetz soils

LOCATION	HORIZON B_1	HORIZON B_2
1	13.01	26.60
2	6.56	21.70
3	21.74	25.42

TABLE 6

Rate of settling after shaking 4 grams of air-dried soil with 400 cc. of distilled water for 24 hours

SETTLING TIME	EXPOSED B_1 HORIZON OF SOLONETZ SOIL (PERCENTAGE OF SOIL IN SUSPENSION)									
	20	121	1x	10	6	8	12	2	4	29x
min.										
0.0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
1.0	37.24	24.26	22.40	35.90	37.04	28.22	17.26	30.12	44.92	49.26
2.5	28.90	17.22	18.60	30.74	24.22	21.60	14.42	26.86	39.74	37.90
5.0	25.36	15.70	15.62	25.70	23.16	18.70	11.24	25.90	37.04	33.82
10.0	20.42	12.54	11.42	23.04	18.76	14.54	7.64	21.96	33.72	32.90
15.0	20.34	11.60	10.44	21.60	12.80	13.60	7.06	21.76	32.80	.....
30.0	16.68	9.80	10.64	17.40	12.64	10.76	6.18	20.16	31.60	30.92
hours										
1	12.52	8.20	7.62	16.26	11.68	8.12	3.62	18.56	30.96	27.42
2	9.82	6.84	5.42	11.38	7.22	5.82	2.90	18.46	28.22	29.36
4	7.46	2.71	5.02	9.54	....	5.22	2.86	18.12	26.64	27.40
6	6.04	....	4.82	7.06	....	3.96	1.94	....	25.18	....
8	5.06	....	4.58	6.70	4.46	....	....	....	....	26.20
24	4.70	2.08	2.78	4.20	2.64	2.60	1.96	8.14	18.30	23.52

SETTLING TIME	EXPOSED A HORIZON OF NORMAL SOIL (PERCENTAGE OF SOIL IN SUSPENSION)									
	19	113	2x	9	5	7	11	1	3	28x
min.										
0.0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
1.0	21.08	11.76	12.64	18.44	25.04	15.32	17.50	10.28	19.86	16.04
2.5	10.14	8.60	8.00	12.66	12.42	10.40	13.48	9.74	13.14	11.90
5.0	6.42	5.08	6.66	7.96	7.24	6.54	8.72	5.56	7.08	5.72
10.0	4.10	4.20	4.80	5.86	5.36	5.16	5.86	5.48	5.70	3.76
15.0	3.18	3.42	3.84	3.88	4.44	5.12	4.44	4.18	3.44	3.42
30.0	2.46	2.64	3.18	3.66	2.96	4.22	3.20	2.76	2.58	2.40
hours										
1	1.44	1.88	2.14	2.44	2.66	3.10	2.58	3.14	2.02	2.36
2	1.66	1.62	2.00	2.14	1.42	2.10	1.90	1.16	1.58	1.52
4	0.98	0.89	1.98	1.22	....	1.68	1.50	1.20	0.74	1.00
6	0.82	....	1.20	....	....	1.90	1.10	1.14	....	....
8	0.98	....	1.04	....	1.32	....	....	....	....	1.14
24	0.80	0.64	1.02	1.12	0.42	1.42	0.74	1.22	0.72	0.72

The dispersion coefficients of the B_1 and B_2 horizons of three badly eroded solonetz profiles were determined as a matter of interest. The results are shown in table 5.

DISCUSSION

The data in the tables indicate the rapidity at which solonetz soils, once the A horizon is removed, may erode. When this stage has been reached these soils have practically no agricultural value, and therefore they should be protected from severe erosion before the B_1 horizon is exposed. Since, however, the A horizon of these soils in this vicinity is only a few inches thick, such soils should be kept in grass as the best means of protection. The grasses growing on these soils where cultivation has not destroyed the native vegetation are principally buffalo (*Buchloe dactyloides*) and blue grama (*Bouteloua gracilis*). Once this soil has been abandoned after having been in cultivation, and while some of the A horizon still remains, triple awn grass (*Aristida oligantha*) soon occupies the area, perhaps largely because this grass requires such a scant amount of plant food for growth. These soils are very low in nitrogen and available phosphorus.

The high dispersion coefficient of the B horizon of the solonetz soils is not the only reason for their rapid erosion. Such areas are usually devoid of, or support only a sparce, vegetative growth and hence there is no buffer against the agitation of rain drops, as there is on the normal soils where the vegetation may offer considerable protection not only in this manner but also because of extensive root development. This not only applies to the solonetz soils where the B horizon is exposed but to those which have been under cultivation at some previous time and when only a thin A horizon remains. This thin A horizon is usually not capable of supporting a vegetative covering that will offer much protection to the soil and, since rain water cannot penetrate the lower horizons, this thin horizon soon becomes supersaturated with water and erodes away rapidly when it occupies sloping areas. Although the clay content is usually somewhat higher, the high active sodium content and the low calcium-sodium ratio of the exposed B horizon of the solonetz profile readily account for the high dispersion coefficients of these eroded areas compared with the surface soil of the normal profile. An approximate estimation of the replaceable sodium may be calculated from the data by subtracting from the sodium in the acetate leachate that which is given as water soluble. The replaceable sodium is high in the B horizon of the solonetz profile. It appears that where the replaceable sodium is high even though there may be considerable water-soluble sodium, and the active calcium is such that the ratio of active sodium to active calcium is approximately 2.00 or less, the soils are unproductive. Such a condition also indicates a soil with a high dispersion coefficient, and, if it occupies an area of much slope, erosion will be quite severe. It will be noted that the average dispersion coefficient for the B horizon of the solonetz profile is 4.29 compared with 0.96 for the surface soils in the normal

profile. Of course the subsoil of the normal soil has a higher dispersion coefficient than the surface soil, but the data given are presented to show the ability which exposed surfaces, as they now occur, have to withstand erosive influences. The data in table 1 record two solonetz samples having exceptionally high dispersion coefficients. Even when these are excluded, the average is 3.28, hence once these soil particles are agitated into suspension they will be carried away by running water to a much greater extent than the surface soils of the normal profile subjected to a similar environment. This is further substantiated by rate of settling data (table 6), which indicate that there is often more solonetz material in suspension after 60 minutes than of normal surface soil after 2.5 minutes of settling. Material having such a high tendency to remain in suspension would be carried a great distance once it became suspended because the current of the run-off water before and after it enters a stream would likely keep the suspension sufficiently agitated to prevent any but a limited amount of settling.

Although both gully and sheet erosion occur on the soils, the type of gully formed through the solonetz profile is quite different from that occurring where the normal profile is encountered. Where gully erosion occurs, very often the gully has its origin in one of the so-called "alkali spots," where it tends to develop a fan-shaped area though it may work back from the solonetz area into the normal soil if the length of the slope is great enough to deliver considerable amounts of water into that particular water channel, and hence the observer may be led astray as to the initial origin of the ditch. Frequently solonetz areas are encountered where there is no well-defined main gully, but a rather broad eroded area where all the surface soil is gone and many small gullies appear, but as these approach normal soil there is a fusion into one gully with steep sides. The walls of the gullies in the solonetz soils are usually sloping and present a very smooth surface, indicating extreme deflocculation. The gully in the solonetz soils grows through the carrying away of this deflocculated material from the broad sloping sides, and by the force of the water working under the broad sides. Eventually the weight of the protruding soil causes it to cave in. In the normal profile, however, the sides of the gully are steep and the widening of the gully is due to exfoliation while the cutting action is confined very largely to the bottom of the ditch. The typical gully in the normal soil is steep-sided, narrow, and deep, whereas in the solonetz soil it is often indistinct, or broad with sloping sides. As the force of the water works under the sides, and caving takes place, this solonetz gully may also exhibit steep sides, especially where the soil mass is cemented together with limestone concretions.

In the control of erosion on lands where both the normal profile and the solonetz profile are encountered, difficulty may be experienced in the building of terraces if the degraded alkali material is used. Rainwater accumulates and stands on the upper side of level terraces or in the depressions in the terrace causeway on solonetz soils, since the degraded material does not permit any

appreciable amount of moisture to penetrate into the lower horizons of the profile. To cite an example, 3 weeks following a rain a composite sample of water was collected from three different pools standing on the exposed B_1 horizon of some solonetz profiles where terraces were still holding. The water was being lost almost entirely by evaporation, was noticeably red as a result of the presence of colloidal material, and upon examination it showed 0.3 per cent of soil in suspension. If the terrace happens to pass through a solonetz area and the degraded material is used in building the terrace, the terrace is weakened at this point because of the deflocculating effect on the soil particles brought about by the presence of large amounts of sodium and the low calcium-sodium ratio in the soil. The terrace tends to melt away, leaving a broad levelled gap as rain falls on such a fill and as water collects behind it. A further study is being made in the use of degraded alkali material as cores and when mixed with the nearby normal soil in the building of terrace fills.

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THE SEPARATION AND IDENTIFICATION OF THE MINERAL CONSTITUENTS OF COLLOIDAL CLAYS[1]

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There has been much diversity of opinion as to the constitution of clays. Most of the early workers regarded kaolinite as the main constituent. Any variation from the kaolinite formula was ascribed to the presence of free silicic acid and free hydrated oxides of iron and aluminum. At the present time there appear to be two viewpoints regarding the constitution of colloidal clays. Mattson (13), in his investigations on the laws of soil colloidal behavior, regards the soil colloids as complexes of indefinite structure and varying composition. He believes them to be amphoteric salts of weak acids (silicic, humic, etc.) and weak bases (chiefly iron and aluminum), formed as a result of the mutual precipitation of electropositive (basic) sols and electronegative (acidic) sols at or near isoelectric conditions (10, 11, 12). This view of the constitution of colloidal clays, although based on more modern conceptions, conforms, in general, to the view held by Van Bemmelen (3). On the other hand, a number of investigators believe that colloidal clays consist for the most part of definite crystalline compounds with stoichiometrical compositions. Especially since the introduction of improved methods and techniques, such as the X-ray, has this view become more widely accepted.

The X-ray studies of Hendricks and Fry (7) indicate that montmorillonite-beidellite, Ordovician bentonite (or a mixture of montmorillonite and quartz), and halloysite are common mineral constituents of soil colloids. Bauxite was found to be present in two of the samples examined. They did not find any evidence of feldspar or mica.

Kelley, Dore, and Brown (8), as a result of their X-ray investigations, concluded that the crystalline materials of bentonites and soil colloids examined by them belong to the montmorillonite-beidellite group of clay minerals, and are essentially crystalline magnesium aluminum silicates in which the magnesium at the surface of the particles has been replaced, giving rise to the exchange

properties.   The magnesium in the interior of the particles is non-exchangeable, but becomes exchangeable when exposed by grinding.

As a general statement, previous work indicates that the main constituents of colloidal clays are free silica, free aluminum and iron oxides, base exchange compounds, and other hydrated aluminum and iron silicates.   The present paper deals with an investigation of methods for the separation and identification of the mineral constituents of colloidal clays.

PREPARATION OF COLLOIDAL CLAYS

The colloidal clays used in this study were prepared from California white and Wyoming yellow bentonites, nontronite, Colby silt loam subsoil, and Vesper sandy loam subsoil.   These materials were free of carbonates and contained little or no organic matter.   The treatment given them, which follows, is similar to that described by Chucka (6).   They were ground to pass a 40-mesh sieve, digested on a steam plate for about 2 hours with N NaCl solution, and then leached in a Büchner funnel with about 500 cc. of N NaCl solution per 25 gm. of material.   The excess NaCl was washed out with approximately 85 per cent by volume ethyl alcohol.   The materials were then dispersed in a dispersion apparatus arranged as suggested by Bouyoucos (4), after which they were suspended in water in the ratio of 25 gm. per 10 liters of water.   After the materials had settled for 24 hours, the supernatant suspensions were siphoned off and passed through a supercentrifuge at such a rate as to produce a colloidal suspension in which the particles were 0.0001 mm. or less in diameter. These suspensions were used in the subsequent experiments.

SEPARATION OF MINERAL CONSTITUENTS BY PHYSICOCHEMICAL METHODS

Separation by specific gravity

A technique suitable for specific gravity separations of fine particles has been perfected by Volk (20) in which tetrabromoethane is used as the heavy liquid. He separated quartz and muscovite from the soil fraction of particle size 0.002 to 0.0003 mm. in diameter.   It was thought, therefore, that this method might be used on the fine clay fraction of particle size 0.0001 mm. and less in diameter, but, under actual trials, separations were not possible because the colloidal material coagulated in the tetrabromoethane.   When bromoform was substituted as the heavy liquid, this coagulation was apparently prevented, but lack of time did not allow completion of tests including centrifuging. The procedure perfected for getting the dispersion is as follows: Coagulate the suspension with NaCl, transfer to centrifuge tubes, and wash free of salt with approximately 85 per cent by volume ethyl alcohol by centrifuging and decanting.   Wash with anhydrous acetone and finally with anhydrous ether. Dry the material at room temperature in a moisture-free atmosphere and then at 70°C. for several minutes to drive off the last traces of ether.   Finally, evacuate and disperse as recommended by Volk, but use bromoform, and then proceed with the centrifuging.

Crystallization in a bomb

Mineralogists have prepared crystalline alumino-silicates by subjecting mixtures of the oxides to high steam pressure. The same procedure has also been used for producing larger crystals from corresponding micro-crystalline compounds. This suggested the possibility of inducing a crystal growth of the micro-crystalline compounds present in colloidal clays, and thus facilitating their separation and identification.

Experiments were conducted in which two white bentonites, a yellow bentonite, and colloids extracted from Vesper sandy loam and Colby silt loam subsoils were subjected to a steam pressure of about 200 atmospheres at a temperature of about 400°C. For this purpose a bomb[3] constructed of special tool steel so as to withstand pressures up to 100,000 pounds per square inch was used. The bomb is a hollow, thick-walled cylinder, $5\frac{1}{2}$ inches long and $2\frac{3}{4}$ inches in diameter. The top half is threaded to fit a heavy bolt. The colloidal clay in gel form was placed in a small platinum crucible, covered, and inserted into the bomb. The bolt was screwed in as tightly as possible, the threads having been covered with graphite to prevent any leaks. The bomb was then placed in a furnace for several days at a temperature of 400°C., which is above the critical temperature of water.

In all cases, upon examination under the microscope after treatment, only quartz crystals, formed probably from the free silica present, were observed. Otherwise, the samples appeared the same as before the bomb treatment, and were found to have the same base-exchange capacities. It is possible that treatment for longer periods (several months), and at higher pressures, might effect crystallization or crystal growth of other constituents.

Electrodialysis

Electrodialysis has been used by many investigators to study exchangeable bases and other plant nutrients. According to Mattson (14), it also offers a means of studying the changes taking place in the colloid complex after desaturation has occurred. In the following experiments, an attempt was made to effect a separation of some of the mineral constituents, especially iron oxide, by prolonged electrodialysis.

A suspension of colloid from yellow bentonite which contained several per cent of iron, and a soil colloid extracted from Colby silt loam subsoil and known to contain free iron oxides were electrodialyzed in a Mattson cell for 72 hours at a potential of 75 volts. The solutions in the outer compartments were renewed every 4 hours for the first 12 hours, and then once every 12 hours thereafter. At the end of 72 hours, the materials were removed and analyzed. They were found to have the same exchange capacities and chemical compositions as previous to electrodialysis, indicating that electrodialysis, carried out

[3] The bomb used was devised by Professor R. C. Emmons.

in this manner, is not a suitable means of separating the main mineral constituents of colloidal clays.

Differential flocculation

Robinson and Holmes (15) attempted to separate colloidal material into fractions of different compositions by fractional coagulation. They evaporated heavy dispersions of soil colloids and found that on concentration a large part of the material coagulated, while the other part remained in suspension. Upon analysis, however, they found no difference in the two fractions, and concluded that separation of minerals of soil colloids could not be effected by this means.

With the same idea in mind, the following experiments were conducted: A suspension of sodium-saturated Colby colloid, which contained about 2 gm. per liter of dry matter, was used. A solution of NaCl sufficiently dilute so as to flocculate only a portion of the suspended material was added. Analyses of the flocculated and unflocculated portions gave identical results, indicating that no separation had been effected. In another experiment the colloid was saturated with barium, washed practically free of excess salt, and dispersed. The suspension was then centrifuged until some of the clay was thrown down. It was thought that perhaps the exchange material saturated with the heavy barium atom would come down first, leaving the non-exchange mineral constituents in suspension. Upon analysis, however, both fractions were found to have the same composition. Another sample of the Colby colloid was saturated with sodium and centrifuged until some of the material came down. Here again, upon analysis, no difference was found between the material thrown down and that remaining in suspension.

The results obtained thus far indicate that differential flocculation is not a suitable means for separating the constituents of colloidal clays.

SEPARATION OF MINERAL CONSTITUENTS OF COLLOIDAL CLAYS BY DIFFERENTIAL SOLUBILITY

Extraction of free silica with sodium carbonate

In order to test the solubility of silica in sodium carbonate, a sample of pure quartz was powdered and separated into fractions of various sizes. These fractions were treated in platinum dishes with different concentrations of sodium carbonate until completely dissolved. The results are summarized in table 1.

These data show that a 2 per cent solution of sodium carbonate is satisfactory for dissolving colloidal silica. This extraction probably also dissolves most, or all, of the free alumina, but has little action on the other constituents. Base-exchange capacity determinations were made on samples of colloids from white bentonite and Colby silt loam before and after treatment with sodium carbonate, and the results are given in table 2.

These results show that boiling with sodium carbonate solution up to as high a concentration as 10 per cent for 12 hours has no appreciable effect on

the base-exchange material. Since the other mineral constituents with the exception of silica and free alumina are probably as resistant as the exchange material, it may be assumed that they were not affected appreciably by the sodium carbonate treatment. The sodium carbonate treatment when properly regulated as regards strength and length of treatment appears, therefore, to be a satisfactory method for separating free silica and possibly alumina.

Some difficulty was encountered in obtaining pure sodium carbonate. Of several brands tried, Merck's Reagent grade anhydrous sodium carbonate proved satisfactory for use without further purification. Ordinary C.P. sodium carbonate may be purified by digesting a concentrated solution in a nickel

TABLE 1

Solubility of finely powdered quartz in various concentrations of Na_2CO_3 solutions

APPROXIMATE SIZE OF QUARTZ PARTICLES	TIME REQUIRED FOR COMPLETE SOLUTION AT BOILING TEMPERATURE		
	2 per cent Na_2CO_3	5 per cent Na_2CO_3	10 per cent Na_2CO_3
mm.	*hrs.*	*hrs.*	*hrs.*
0.003 to 0.0006	36	36	36
0.0006 to 0.0003	24	22	14
0.0003 to 0.00015	12	12	8
< 0.00015	10	8	6

TABLE 2

Effect of Na_2CO_3 treatment on the base exchange capacities of a bentonite and a soil colloid

SAMPLE	BASE-EXCHANGE CAPACITIES PER 100 GM. OF CLAYS UNTREATED AND TREATED WITH SODIUM CARBONATE			
	Untreated	Boiled 12 hours with sodium carbonate		
		2 per cent	5 per cent	10 per cent
	m.e.	*m.e.*	*m.e.*	*m.e.*
Bentonite.............................	147	145	149	147
Colby colloid..........................	98	96	96	98

container for 24 hours and filtering off the coagulated impurities. After this treatment is repeated, the salt is recrystallized. It should now be pure and ready for use.

Extraction of free iron oxide with sodium acid oxalate

The strength of HCl required to dissolve the free iron oxides of soil colloids at a reasonable rate is so great as very seriously to attack the other constituents. Oxalic acid is commonly recognized as a good solvent for iron oxides. Robinson and Holmes (15) found that, in addition to iron oxide, considerable amounts of combined alumina and silica were dissolved from colloids by rather strong oxalic acid at an elevated temperature.

Tamm (18) used acid ammonium oxalate in an attempt to separate the soil colloids from the rest of the soil, the idea being that the iron oxides, silica, and alumina existing in the colloidal state would dissolve.

In the following experiments, a solution of sodium acid oxalate, 0.1 N with respect to both sodium oxalate and oxalic acid, was used. One-half gram samples of Colby colloid and Vesper colloid were shaken intermittently with 300 cc. of the oxalate solution at room temperature for 3 days. The suspensions were coagulated, and the material was washed several times with ammonium acetate and then treated with 2 per cent sodium carbonate solution to dissolve any free silica. Base-exchange capacities of the samples were then determined, after which total analyses were made. The results, together with those of analyses of untreated samples, are summarized in table 3.

TABLE 3

Analyses of Colby and Vesper colloids, untreated and treated with sodium acid oxalate and sodium carbonate

	AMOUNTS OF CONSTITUENTS AND BASE-EXCHANGE CAPACITIES			
	Colby colloid		Vesper colloid	
	Untreated	Treated	Untreated	Treated
	per cent	*per cent*	*per cent*	*per cent*
SiO₂	56.1	60.0	54.8	55.7
Al₂O₃	24.1	27.0	26.6	27.3
Fe₂O₃	14.9	8.5	7.3	6.3
K₂O	0.7	0.7	4.5	4.7
MgO	2.9	3.1	3.1	3.2
Total	98.7	99.3	96.3	97.2
	m.e.	*m.e.*	*m.e.*	*m.e.*
Base-exchange capacity per 100 gm	98	154	53	60

In the case of the Colby colloid treated with oxalate solution, there was a significant decrease in the percentage of Fe_2O_3 and a corresponding increase in SiO_2 and Al_2O_3, while the potassium and magnesium content increased only slightly. The untreated colloid was reddish brown in color, while the treated sample was yellowish gray, similar to the color of the iron silicate, nontronite. This indicated that practically all of the free iron oxides were removed by the oxalate treatment, and the iron remaining was in the form of iron silicate. The removal of the iron oxides resulted in a large increase in base-exchange capacity, even when calculated on the basis of the weight before removal of the iron. This suggested that the iron oxide was either physically or chemically bound up with the base exchange material, thereby reducing the base exchange capacity. Lundblad (9), after treating soil colloids with ammonium oxalate, also found increases in base capacities.

The Vesper colloid, on the other hand, had much the same composition and base-exchange capacity after treatment as before. The color of the untreated colloid was a yellowish gray both before and after treatment. It was concluded that the Vesper colloid contains a relatively small amount of free iron oxides, most of the iron being present probably as a silicate.

A colloid separated from a Hawaiian laterite known to contain large amounts of iron oxides was treated with the oxalate solution. The color before treatment was a dark brown, whereas after treatment it was almost white. The iron oxide content was reduced from 44.2 per cent to 3.4 per cent. This was a striking example of the solvent action of the oxalate solution on iron oxides.

Samples of colloids prepared from the iron silicate, nontronite, and a yellow bentonite, were unchanged in color, composition, and base-exchange capacity after treatment with the oxalate solution, indicating that no free iron oxides were present.

In all of the foregoing experiments there was some solution (5 to 20 per cent) of material other than the iron oxides, but from the analyses there was no indication of solution of any definite compound other than the iron oxides. It is concluded that, if properly regulated, treatment of colloidal clays with sodium acid oxalate, although not ideal, may be used to advantage in certain cases for separating the free iron oxides present.

Solubility in hydrofluoric acid

Preliminary tests indicated that some silicate minerals are attacked by HF much more readily than others. Finely powdered talc dissolved almost instantly in 2.5 per cent HF (approximately 1 cc. concentrated 48 per cent reagent diluted to 20 cc.), whereas muscovite and tremolite did not dissolve appreciably in 10 minutes. This suggested that the mineral constituents of colloidal clays might have a differential solubility in dilute HF.

A sample of hydrogen-saturated white bentonite gel containing 0.2 gm. of dry material was treated in a platinum dish for 1 minute with 50 cc. of 0.5 per cent HF by weight, and the suspension filtered with suction by means of a paraffined Gooch crucible fitted with a pad of filter paper pulp and held in a paraffined suction flask. This filtration took about 2 minutes. The residue was washed 10 times with 0.05 N HNO$_3$ and analyzed. It was found that, although an appreciable amount of the material had dissolved, the composition of the residue was the same as that of the original material.

It seemed possible that the bentonite used contained only one mineral, and hence, samples of Vesper colloid and Colby colloid, known to contain different constituents, were subjected to the same treatment. In both cases, appreciable quantities of material were dissolved, but the analyses of the residues and untreated samples gave identical results. It was concluded that the mineral constituents of colloidal clays do not have a marked differential solubility in HF, probably because the clay is so finely divided that, in a strong solvent like HF, differences in rate of solubility are difficult to measure.

Extraction with ammonium acetate after fine grinding

It was thought that if the minerals present in colloidal clays were ground fine enough in a ball mill, some of them might become soluble in a weak solvent like ammonium acetate solution. Since the crystals of the base-exchange compounds presumably are sufficiently porous to allow reactions with solutions to go on internally as well as at the surface, their solubility should not be increased markedly by grinding. Smolik (17), and Kelley, Dore, and Brown (8) have reported marked effects of grinding soil colloids and bentonites on the exchangeability of otherwise non-replaceable bases.

A sample of purified white bentonite, ammonium-saturated, containing 7.3 per cent of non-replaceable MgO, was ground for 90 hours in a steel ball mill provided with chrome alloy steel balls. At the end of the run, the sample was found to be highly contaminated with iron from the mill. A weighed portion of the ground sample was leached with neutral N ammonium acetate and the leachate analyzed. It was found that a large portion of the non-replaceable magnesium present in the original sample, together with some silica and a little alumina, had become soluble in the ammonium acetate.

In order to eliminate contamination as much as possible, efforts were made to obtain a more resistant mill. A porcelain-lined bowl was found to be unsatisfactory, 3 gm. of material being ground off the bowl during a 2-day run. A bowl made of high quality carbon tool steel proved to be quite satisfactory. It was specially hardened and tempered so as to be highly resistant to abrasion. The cover of the bowl was provided with a three-fourths inch hole for the purpose of replacing the atmosphere in the bowl with dry air so as to lessen the tendency of the material to stick to the sides of the bowl and to the balls, thus making the grinding more effective. The hole was closed with a threaded steel stopper.

A sample of purified ammonium-saturated white bentonite was dried at 150°C. for 24 hours and then ground in the ball mill for 72 hours. It was then leached with ammonium acetate and the leachate analyzed. About 15 per cent of the non-exchangeable magnesium present and 1 per cent of the silica were extracted, but tests for soluble aluminum and iron were negative. This indicated that a magnesium silicate was being made more soluble by the grinding. The sample was then treated with sodium acid oxalate to remove any free ferric oxide arising by contamination from the mill, and finally with Na_2CO_3 to remove any free silica. A base-exchange capacity determination agreed with the base-exchange capacity of the original material.

Kelley, Dore, and Brown (8) believed that the non-replaceable bases are made replaceable by means of grinding. This would imply that the base capacity would be increased by an amount equal to the amount of base made replaceable. The preceding experiment, however, did not bear this out, but rather suggested that other minerals than the base-exchange compounds were being affected by the grinding treatment. Further grinding experiments with other clays are now being carried on.

IDENTIFICATION OF MINERAL CONSTITUENTS OF COLLOIDAL CLAYS

Identification of an iron silicate as a mineral constituent

From the preceding experiments and from the work of other investigators, it appears that not all of the iron present in colloidal clays is in the oxide form. The existence of an iron silicate mineral in clays has been suggested by Robinson and Holmes (15), and the iron silicate, nontronite, has been identified in soils (1, 5) with a petrographic microscope. Unfortunately, petrographic methods have not been adapted to the study of particles finer than 0.005 mm. in diameter.

There is no agreement as to the exact composition of nontronite, some ascribing to it the formula, $Fe_2O_3 \cdot 2SiO_2 \cdot 2H_2O$, and considering it to be the iron isomorph of kaolinite, $Al_2O_3 \cdot 2SiO_2 \cdot 2H_2O$; others give $Fe_2O_3 \cdot 3SiO_2 \cdot XH_2O$ as the formula. One of the difficulties involved is obtaining pure samples.

TABLE 4

Analyses of purified colloids from nontronite and bentonite

SAMPLE	SiO_2	Fe_2O_3	Al_2O_3	MgO	$\dfrac{SiO_2}{Fe_2O_3 + Al_2O_3}$	BASE-EXCHANGE CAPACITY PER 100 GM.
	per cent	*per cent*	*per cent*	*per cent*		*m.e.*
Nontronite no. 1........................	57.0	38.9	0.8	2.3	3.9	127
Nontronite no. 2........................	55.3	41.3	0.4	1.1	3.6	144
White bentonite no. 2....................	67.2	1.4	28.1	3.7	3.9	139

Colloidal suspensions prepared from two different samples of nontronite obtained from the Woody district, California, were treated with sodium carbonate to remove any free silica and alumina. Base-exchange capacities were determined on the two samples, and total analyses made. The results are summarized in table 4, together with an analysis of a purified white bentonite.

These data confirm the idea of the existence of an iron exchange compound similar in nature to the aluminum exchange compound. However, the analyses indicate that the ratio of SiO_2 to Fe_2O_3 of the compound is 4 to 1 rather than 3 to 1 or 2 to 1. The presence of MgO shows that the material is probably not altogether pure, which might account for the variation from the ratio of 4 to 1. Table 4 shows that the base-exchange capacities of the purified nontronites agree fairly well with the base-exchange capacity of the bentonite.

An X-ray pattern obtained of one of the purified nontronite samples is very similar to that of a purified white bentonite.

The color of the Vesper and Colby colloids after treatment with sodium acid oxalate to remove free iron oxides approached that of nontronite, giving further credence to the idea that an iron silicate-exchange compound similar to

the aluminum-exchange compound is present in some colloidal clays containing iron other than that in the oxide form.

Fixation of cations by wetting and drying

Besides free silica, free oxides of aluminum and iron, and the iron and aluminum base exchange compounds, there are probably other intimately mixed minerals in most colloidal clays. This is indicated by the grinding experiments. It was thought that further evidence of this might be obtained by a study of the effect of wetting and drying of colloidal clays in the presence of salts, since Volk (21) found that by alternate wetting and drying of soils in the presence of KCl potassium is fixed by the clay fraction of soils in a form not extractable with ammonium acetate; and since an artificial mixture of

TABLE 5

Analyses of purified colloids from yellow bentonite and Colby silt loam subsoil, both untreated and wetted and dried 40 times in the presence of KCl

	AMOUNTS OF CONSTITUENTS AND BASE-EXCHANGE CAPACITIES			
	Yellow bentonite		Colby colloid	
	Untreated	Wetted and dried 40 times with KCl	Untreated	Wetted and dried 40 times with KCl
	per cent	*per cent*	*per cent*	*per cent*
SiO$_2$	64.9	63.8	56.1	55.4
Al$_2$O$_3$	27.6	26.8	24.1	23.7
Fe$_2$O$_3$	4.0	3.9	14.9	14.6
MgO	2.8	2.9	2.9	2.7
K$_2$O	Trace	1.5	0.7	2.0
Total	99.3	98.9	98.7	98.4
	m.e.	*m.e.*	*m.e.*	*m.e.*
Base-exchange capacity per 100 gm	116	118	98	96

silica gel, aluminum hydroxide, and calcium hydroxide did not possess this fixing ability, he concluded that the potassium reacts with a silicate mineral to form a difficultly soluble potassium compound, which he believed to be muscovite.

It was decided to test the fixing power of purified colloidal clays in relation to their base-exchange capacities in order to determine whether it was the base-exchange material or some other mineral that reacted with the potassium. Samples of a purified yellow bentonite and a Colby colloid were saturated with potassium and alternately wetted and dried 40 times at 70°C. in the presence of excess KCl. The samples were then extracted with ammonium acetate to remove any soluble and exchangeable potassium present. Base-exchange capacities were determined and total analyses made. The results, together with those of analyses of untreated samples, are given in table 5.

These data show that a considerable amount of potash was fixed by the clays with no significant change in the base-exchange capacities. If the potash had reacted with the exchange compounds to form an insoluble potassium aluminum or iron silicate, one would expect a reduction of the base-exchange capacity. Therefore, it was concluded that there are other minerals present besides the exchange compounds which react with the potassium. As further evidence of this, it was found that when the clays were saturated with potassium and wetted and dried in the absence of any excess KCl, no potassium was fixed, nor was there any decrease in the base-exchange capacity. This showed that the potassium in the exchange form does not react further with the base-exchange material.

Samples of yellow bentonite saturated with Na, Ca, and Mg were also wetted and dried in the presence of excess NaCl, CaCl₂, and MgCl₂ respectively. It was found, however, that none of these cations were fixed, and no effect on the base-exchange capacity was noted. Apparently, potassium is the only cation studied which can be fixed by the clay minerals.

Possible use of ultra-violet microphotographs

If the individual particles of a colloidal suspension could be photographed, some indication of their differences in shape and other properties might be obtained, and hence their identity established by comparison with known minerals. Work in other fields has indicated the possibility of photographing particles of approximately 100 $\mu\mu$ in diameter by the use of the ultra-violet microscope. This suggests its use for studying colloidal clays. Preliminary tests on the absorption spectra of some colloidal clays indicate that clays absorb ultra-violet light of the wave length used with the microscope, and, therefore, there is some hope that the ultra-violet microscope might be suitable for the study of clays. Unfortunately, the apparatus was not available for use at the time of this investigation.

Loss of water at elevated temperatures

If it could be shown that the percentage losses of water from colloidal clays at elevated temperatures are related to the losses from certain minerals at these same temperatures, this would indicate the presence of these minerals. Accordingly, samples of two different bentonites, variously saturated, and a sample of finely powdered sericite $(H,K)AlSiO_4$, were heated successively at higher temperatures in a muffle furnace. Since ferrous iron was absent, the actual loss of water should be represented by the loss in weight. The loss in water at the different temperatures was calculated in percentages of the weight of the sample after being heated at 100°C. for 24 hours. The results are given in table 6.

In the case of the bentonites, there is a distinct maximum loss of water between 550° and 650°C. This temperature interval corresponds to that at which the base-exchange material loses its base-exchange properties, as shown by Kelley, Dore, and Brown (8), and since the bentonites are largely composed

of base-exchange material, this water loss can be ascribed to that substance. Baver and Horner (2) also obtained a maximum loss in water at about this same temperature range. There is some indication of a second rise in loss of water at 850°C. in the case of the white bentonite and an initial rise at 350° and 450°C. in the case of the yellow bentonite, which may be due to the presence of minerals other than the exchange material. Since the sericite shows no distinct maximum, but rather a uniform loss over the temperature range studied, its presence in the bentonites is not demonstrated. Obviously, before any definite conclusions regarding this method of identifying the clay constituents are drawn, other finely powdered minerals in pure form should be tested.

TABLE 6

Loss of water by bentonites and sericite on heating for 24-hour periods successively at temperatures indicated

TEMPERATURES OF SUCCESSIVE 24-HOUR PERIODS THAT SAMPLE WAS HEATED	WATER LOST DURING EACH HEATING PERIOD, BASED ON WEIGHT AFTER HEATING AT 100°C. FOR 24 HOURS						
	White bentonite			Yellow bentonite			Sericite
	H-satu-rated	Na-satu-rated	Ca-satu-rated	H-satu-rated	Na-satu-rated	Ca-satu-rated	
°C.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
250	0.46	0.00	0.30	0.73	0.00	0.31	0.46
350	0.88	0.23	0.95	0.74	0.32	1.11	0.40
450	1.09	0.46	0.66	1.10	1.11	0.67	0.73
550	1.09	1.66	1.31	0.92	1.75	1.18	0.53
650	2.29	2.49	3.14	2.93	3.50	3.87	0.72
750	0.59	0.83	0.66	0.92	0.41	0.78	0.40
850	0.75	1.57	1.09	0.55	0.49	0.56	0.13
950	0.33	0.37	0.00	0.00	0.00	0.00	0.07

Chemical analyses and stoichiometrical calculations of results to fit probable minerals

Total analyses were made on two different bentonites and two soil colloids after the removal of free silica, alumina, and iron oxide. The data are given in table 7.

If the exact composition of the exchange material, the magnesium compound, and the potash compound were known, it would be possible to allocate stoichio-metrically the results of the chemical analysis to definite minerals, and thus present a complete picture of the minerals in soil colloids.

From previous work, it appears that the base-exchange material probably has a SiO_2 to R_2O_3 ratio of 4 to 1. Since Volk (20) found muscovite in the fraction of soil 0.002 to 0.0003 mm. in diameter, it may be assumed for trial purposes that the potash present in colloidal clays can be ascribed to muscovite, $K_2O \cdot 3Al_2O_3 \cdot 6SiO_2$. The grinding experiments indicated the presence of a magnesium silicate in the white bentonite, and for trial purposes the mag-

nesium present in colloidal clays may be ascribed to talc, a hydrated secondary magnesium silicate. The analysis of an ignited sample of talc corresponded to the formula $4MgO \cdot 3SiO_2$. The iron is probably present as an iron silicate exchange compound.

Using the results of the chemical analyses reported in table 7, calculations were made in which the potash present and stoichiometrical amounts of alumina and silica were allocated to muscovite, and its percentage was calculated. Similarly, the iron and necessary silica were allocated to an iron silicate exchange compound having a SiO_2 to Fe_2O_3 ratio of 4 to 1; the magnesium and the necessary silica, to talc. The silica and alumina remaining presumably made up an aluminum exchange compound; its percentage and silica to alumina

TABLE 7

Chemical composition of bentonites and soil colloids after removal of free silica, alumina, and iron oxides, and mineral composition based on stoichiometrical allocations of chemical constituents to minerals assumed to be present

	CHEMICAL AND MINERAL COMPOSITION OF BENTONITES AND SOIL COLLOIDS									RATIO $\frac{SiO_2}{Al_2O_3}$ OF ALUMINUM EXCHANGE COMPOUND	BASE-EXCHANGE CAPACITY PER 100 GM.
	Percentages of oxides by total analysis					Percentages of minerals by stoichiometrical calculation					
	SiO_2	Al_2O_3	Fe_2O_3	K_2O	MgO	Talc	Muscovite	Iron exchange compound	Aluminum exchange compound by difference		
											m.e.
White bentonite no. 1....	67.5	22.4	1.65	0.0	7.3	21.2	0.0	4.2	74.6	3.9	160
Yellow bentonite.........	65.9	26.6	4.0	0.0	2.6	7.8	0.0	10.4	81.8	3.6	120
Colby colloid............	60.0	27.0	8.5	0.7	3.1	9.0	5.9	21.2	63.9	2.7	154
Vesper colloid...........	56.7	26.0	6.3	4.7	2.8	8.3	37.9	16.2	37.6	3.6	60

ratio are reported. The results of all of these assumptions and stoichiometrical calculations are given in table 7.

If it is assumed that the ratio of silica to alumina in the aluminum exchange compound is 4 to 1, then, in the case of the white bentonite the assumptions and results of the calculations fit the chemical analyses very closely; in the case of the yellow bentonite and Vesper colloid reasonably well, but not so satisfactorily in the case of the Colby colloid.

It should be noted that the exchange capacities of the four samples correspond reasonably well with the sum of the iron and aluminum exchange materials present, with the exception of the yellow bentonite, in which case the exchange capacity is low. Variations of the ratio of silica to alumina from

the assumed value, and irregularities in the exchange capacities may be accounted for by imperfect allocations of the constituents to minerals. It is possible that some of the iron and magnesium should be allocated to mica, and that small amounts of minerals other than those mentioned may be present. Obviously, additional information is needed before a satisfactory picture of the mineral composition of colloidal clays can be drawn.

Attempts at preparation of the aluminum base exchange compound

Since the aluminum exchange compound is often the most important constituent of colloidal clays, it would be extremely helpful if its exact composition were known. A number of artificial alumino-silicates possessing base-exchange properties have been prepared, but none of these have been found to be similar in certain respects to the base-exchange material found in colloidal clays.

The base-exchange compound of bentonites has been shown by several investigators to be similar to that found in soils. It has been suggested (16, 19) that these bentonites were formed from feldspathic volcanic ash which was deposited in water or submerged after deposition. Presumably, hydrolysis took place rapidly, and the water became alkaline, about pH 8.4. The feldspathic ash under these conditions decomposed, giving rise to the bentonites containing large amounts of base-exchange material.

Attempts were made to prepare the base-exchange material by subjecting finely powdered albite to alkaline weathering treatments. The material was leached and digested with various concentrations of Na_2CO_3 and $NaHCO_3$ at different temperatures for various lengths of time up to several weeks. In some cases, compounds were formed which possessed high base-exchange capacities, but they were found to decompose easily in dilute acids, indicating that they were similar to the common artificial zeolites and were not the true soil base-exchange compounds. In no instance was there any indication of the formation of a base-exchange compound similar to that found in soils.

Since colloidal clays show properties similar in some respects to finely powdered mica, they might possibly arise from this mineral under certain weathering conditions. Laboratory experiments to test this possibility are suggested as a promising field for further research.

SUMMARY

The purpose of this study was to investigate some of the possibilities of separating and identifying the mineral constituents of colloidal clays. The results may be summarized as follows:

Colloidal clays were subjected to steam pressure of about 200 atmospheres in a bomb for several days. The colloidal silica crystallized out as quartz, whereas the other constituents were unaffected.

Prolonged electrodialysis and attempts to flocculate colloidal clay suspensions differentially proved unsuccessful as means for separating the clay constituents.

A 2 per cent solution of sodium carbonate at boiling temperature for several hours dissolves free silica from colloidal clays without affecting the base-exchange material. Prolonged treat-

ment in the cold of colloidal clays (known to contain free iron oxides) with a solution of sodium acid oxalate (0.1 N with respect to sodium and hydrogen) removed the free iron oxides, but also dissolved other constituents to some extent. No differential solubility of the constituents of colloidal clays in dilute HF was noted.

Grinding clays in a ball mill followed by extraction with ammonium acetate and other solvents offers much promise as a means of separating some of the clay constituents. Preliminary experiments indicate that a magnesium silicate may be extracted from bentonite, while the exchange material is unaffected.

Further evidence of the existence of an iron silicate exchange compound is presented. It is shown to be similar in composition and nature to the aluminum exchange compound.

Colloidal clays fixed considerable amounts of potassium in a form not exchangeable and having no effect on the base-exchange capacity, indicating the presence of minerals other than the base-exchange compounds.

Total analyses were made of some colloidal clays after the removal of free silica, alumina, and iron oxide, and stoichiometrical allocations of the constituents were made to fit the minerals thought likely to be present. In three cases out of four the data fitted the assumptions reasonably well, indicating the possible presence of talc, muscovite, and the exchange compounds having a SiO_2 to R_2O_3 ratio of 4 to 1.

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BOOK REVIEWS

A Textbook of Organic Chemistry, Third Edition Revised. By JOSEPH SCUDDER
CHAMBERLAIN. P. Blakiston's Son & Co., Inc., Philadelphia, 1934. Pp.
xxv + 873, tables 17. Price $4.00.

In his Preface to the Third Edition, the author notes that "So far as the
present revision affects the rest of the text there have been a few changes intro-
duced in order to agree with modern electronic theories and with the latest
researches in the field of carbohydrate chemistry. The chemical and physical
conditions under which reactions take place have also been added, where pre-
viously omitted."

The new edition has in part been condensed, and changes have been made in
the arrangement of the subject matter. The book is made up of three parts;
namely, Part I—A-Cyclic Compounds, Aliphatic Series; Part II—Cyclic Series;
and Part III—Supplementary Topics. Pages 801 and 802 contain a list of
books of reference, and pages 803 to 842, inclusive, comprise two appendixes.

In making the revision, the author was guided by his teaching experience and
by comments and suggestions received from various sources. Altogether, he
has been fortunate in developing a text which will be even more useful than it
has been in the past. Teachers of organic chemistry, as well as students, will
find this text both helpful and useful.

Perkin and Kipping's Organic Chemistry, Part III, Entirely New Edition. By
F. STANLEY KIPPING and F. BARRY KIPPING. W. & R. Chambers, Ltd.,
Edinburgh, 1934. Pp. viii + 342.

This is a new edition of *Perkin and Kipping's Organic Chemistry.* The
authors state that "The present volume has been written as a continuation of
Part I and II of Perkin and Kipping's Organic Chemistry, and is intended
mainly for the use of students who are working for an Honours Degree Exami-
nation. It is hoped that it may also be helpful to teachers, and to others who
are interested in the more recent developments of certain branches of organic
chemistry."

There are 21 chapters in Part III. The numbering of these is a continuation
of that for Parts I and II. The topics dealt with in the several chapters of
Part III are: The Electronic Formulae of Organic Compounds; The Physical
Properties of Organic Compounds; Geometrical Isomerism; Geometrical
Isomerism of the Oximes and other Compounds of Tervalent Nitrogen; Optical
Isomerism; Optically Active Compounds of Nitrogen, Tin, Silicon, Sulphur,
etc.; Cycloparaffins and Cyclo-olefines; Olefinic Compounds; Ketones, Ketonic
Acids, and Ketenes; Isomeric Change; The Configurations, Synthesis, and

Glucosidic Structures of the Monosaccharides; Disaccharides and Polysaccharides; The Monocyclic Terpenes and Related Compounds; Dicyclic Terpenes and Related Compounds; Sesquiterpenes, Open-chain Terpene Derivatives, and Rubber; Cartenoids, Pyrones, Anthocyanins, and Depsides; The Structure of Benzene, and Aromatic Substitution; The Orientation of Benzene Derivatives; Polycyclic Hydrocarbons; Alkali Metal Compounds and Some Derivatives of Arsenic; Compounds in which Elements Show Abnormal Valencies; Steric Hindrance; and Heterocyclic Compounds. The Index covers the three parts.

The authors have made a distinct contribution to the resources of the teacher of organic chemistry. As they note in the Preface: "Whether our selection of the subjects included in this book is the best or otherwise is no doubt a matter of opinion, but it is based on the results of many years of experience in teaching this branch and grade of chemistry."

A Laboratory Manual for the Chemical Analysis of Water and Sewage. First Edition. By E. F. ELDRIDGE and F. R. THEROUX. Edwards Bros., Inc., Ann Arbor, Michigan, 1935. Pp. iii + 201.

The forward march of sanitation has made us realize the significance of water supplies and sewage disposal in the organization and functioning of modern society. Every municipality, to say nothing of the larger political divisions of the United States, is obliged to give more and more thought to methods and procedures for assuring its population of a satisfactory supply of potable water. Good water for domestic and industrial uses and effective methods for the disposal of domestic and trade wastes are matters of general concern. Hence, the need for reliable laboratory manuals dealing with the chemical analysis of water and sewage! The present volume, aside from the Preface, is made up of three sections and an appendix. Section I deals with methods of analysis; Section II, with reagents and standard solutions; and Section III, with chemistry and discussion of methods.

The authors note that "The book contains specific directions in outline form for making the chemical determinations necessary for the control of water and sewage treatment plants, the analysis of polluted water, and the examination of industrial wastes. Each determination is accompanied by calculation formulae, many of which are numerically illustrated." The authors also point out that the manual will be useful to water and sewage treatment plant operators, plant chemists, sanitary engineers, and instructors in our colleges and universities.

Practical Methods in Biochemistry. By FREDERICK C. KOCH. William Wood & Company, Baltimore, 1934. Pp. 7 + 282, figs. 17, tables 35. Price $2.25.

In the rapidly expanding field of biochemistry, there are many questions which constantly call for the devising of new methods of study and for the

improvement of laboratory technic. The author has rendered a service to chemists in having brought together the information given in *Practical Methods in Biochemistry*.

"The beginner in biochemistry," says the author, "should have good fundamental training in chemistry, physics, and general biology. Chemistry is so important that the preliminary work should include a good course in the theoretical and practical aspects of quantitative analysis, a working knowledge of the application of the fundamental laws of physical chemistry to solutions and gases, and the mastery of the more important fundamental facts and concepts of organic chemistry. Biochemistry is the application of these laws, concepts, facts, and methods to the study of any field in biology. Every biological activity involves some chemical change in one or more constituents in the system under consideration."

The three parts of the book are entitled, respectively, The Chemistry of Cell Constituents; the Chemistry of the Digestive Tract; and Blood and Urine. In Part I, we have chapters on carbohydrates, lipins, proteins, nucleoproteins and nucleic acids, and hydrogen ion concentration. The chapters in Part II are designated; respectively: Salivary Digestion; Gastric Digestion; Intestinal Digestion; and Bile. The chapters in Part III are entitled: Blood and Hemoglobin; The Quantitative Analysis of Blood; The Quantitative Analysis of Urine; and The Chemical Examination of Urine for Pathological Conditions. Pages 221 to 274 are devoted to the appendix, which has to do with general laboratory instructions and contains much specific and useful information for the laboratory worker.

Bergey's Manual of Determinative Bacteriology. By DAVID H. BERGEY. Williams & Wilkins Company, Baltimore, 1934. Pp. xvi + 664.

This is the fourth edition of a book widely known and widely used. The successive revisions have undoubtedly added value to the book. The author has been assisted by a committee of the Society of American Bacteriologists. This committee consisted of Robert S. Breed, Frank M. Huntoon, Bernard W. Hammer, E. G. D. Murray, and Francis C. Harrison. "The Committee on Manual," states in the Preface to the Fourth Edition that "The interest in the Manual by bacteriologists has increased greatly, during the past two years, not only on the part of those in the United States but also those in other parts of the world, as indicated by the number of reprints of descriptions of new organisms sent to the Committee by the authors, and also by numerous helpful suggestions for the improvement of the Manual." New genera have been recognized and descriptions of about 50 new species have been included in the book.

The Introduction is followed by suggestions for the use of the Manual in classifying unknown organisms; abridged key of the bacterial families, tribes, and genera; how bacteria are named and identified; bacteriological code; classification of the organisms as arranged in six orders, and the corresponding

numbers of families, tribes, and genera. The authors are to be commended for the effective arrangement of the subject matter and for the earnest and successful effort to provide the greatest accuracy in the descriptions of the several organisms. As they note in the Introduction: "No organism can be classified before we have determined, through detailed study, its morphologic, cultural and pathogenic characters."

One can readily understand why the earlier editions of the book have been so popular, not only in the United States but also in foreign countries. It constitutes a reference work which should be on the shelf of every worker directly or indirectly concerned with bacteriological technic.

Plant Life, A Textbook of Botany. By D. B. SWINGLE. D. Van Nostrand Company, Inc., New York, 1935. Pp. xiv + 441, figs. 290. Price $3.00.

The author has made a distinct contribution to the teaching and reference resources of our botanists. As he points out: "The student will find the subject of botany presented in this book in a somewhat different way from that commonly used. The prevailing tendency is for a morphological approach, the consideration of function being incidental. The result is that students find their textbooks heavy and dull, and they get little comfort from the fact that trained botanists see them teeming with interest. . . . . In this text an advance is attempted in the method of presentation. Use is made of the fact that our interest in plants and animals centers around life processes—methods of obtaining and utilizing food, methods of avoiding the dangers that beset them on every hand, and the ways by which they are perpetuated through endless generations. Activities in which the plant participates constitute the central theme, and structures are described as mechanisms by which these activities can be carried out."

The Preface and the Introduction are followed by eight parts, entitled, respectively: The Living Plant; Plants and Their Surroundings; Plants and Their Food; The Growth of Plants; Reproduction in Plants; The Different Kinds of Plants; Plants Past and Present; and Relation of Plants to each other and to Animals. In writing the book, the author has been painstaking as to detail and has succeeded in preparing an attractive as well as a useful volume. The publishers are to be commended for the quality of their part of the work.

The Theory and Practice of Silviculture. By F. S. BAKER. McGraw-Hill Book Company, Inc., New York, 1934. Pp. xiv + 502, figs. 87, tables 51. Price $5.00.

Silviculture has always been a subject of major economic interest. Its importance has been emphasized in recent years by the more intelligent planning of the use of land resources in different countries. In the United States particularly the withdrawal of large areas of land from agricultural production has called attention to the need for more careful planning in the use of our lands now in forests or to be reserved for the growing of forest trees. Trees like

cultivated crops, have their insect enemies and fungous diseases. They have as distinct a relation to the soil as do cultivated crops. The growing of trees is, therefore, deservedly a popular subject.

We are told by the author that "Readers who are familiar with other works on silviculture, both American and European, will find the arrangement of the early chapters unusual and perhaps confusing, for it has long been customary to discuss chapter by chapter the climatic and edaphic factors and their effects upon the processes of growth reproduction and so on. The arrangement has been revised here. Physiological processes are discussed primarily and note is made of how each is affected by varying different site factors."

Chapter I, which follows the Preface, constitutes an introductory statement. This, in turn, is followed by Parts I to V, entitled, respectively: Plant Physiology; Forest Ecology; Systematized Silvicultural Experience; The Forest Itself as a Source of Silvicultural Knowledge; and Silvicultural Literature. The designations of the chapters in Part I are: Forest Genetics; The Water Cycle; The Carbon Cycle—Photosynthesis; The Nitrogen and Mineral Cycles; Growth of Trees; Reproduction of the Forest; and Injury, Disease and Death of Forest Trees. In Part II, we find chapters on Forest Types and Sites; Form and Composition of Stands; Density of Stands; Tolerance; The Theory of Succession; and Crown Classification. The third part is made up of chapters on Silvicultural Systems Depending on Seed for Reproduction; Coppice Forests; The Analysis of Silvicultural Systems, and Intermediate Cuttings. There is only one chapter in Part IV, entitled: Field Studies in Silviculture. Part V contains two chapters, entitled Applied Silviculture and Applied Silviculture— Western United States, respectively.

The book represents a distinct and valuable contribution on the subject of silviculture. Both the author and publishers are to be commended for having done their work well.

A Study of the Sediments of the North Baltic and Adjoining Seas. By STINA GRIPENBERG. Helsinki-Helsingfors, 1934. Pp. 231, tables 28, figs. 45.

The data reported in this volume are of distinct interest to the specialist as well as to the more general reader. The scope of the work is indicated in the Preface as follows: "In 1924 Professor Rolf Witting, Director of the Thalassological Institute of Helsingfors, suggested that I should undertake the examination of a number of bottom samples which were to be collected from the Gulf of Finland, the Gulf of Bothnia and the North Baltic during the yearly cruises of the s/s Nautilus. This suggestion was followed, and the results of the investigation are set forth in the present paper. About a hundred and thirty samples, collected during the years 1924–1930, were examined. The chief value of an investigation of this kind, apart from a mere knowledge of the character of the sediments, is its bearing on the early history of the Baltic Sea. In this respect, the results presented are only to be regarded as a contribution to the subject, since an examination allowing of more definite and detailed conclusions

would have demanded not only chemical and mechanical, but also minera-
logical and biological analyses of the samples."

The nature of the contents of the treatise is indicated by the designation of
the chapters, namely: Introduction; Chemical Analysis; Mechanical Analysis;
Preliminary Treatment; The Samples; and Discussion. The Bibliography
contains 136 titles. Among others, the students of soil and soil erosion will
find much of interest in this report.

A Program for Land Use in Northern Minnesota. By OSCAR B. JESNESS,
REYNOLDS I. NOWELL, and associates. The University of Minnesota Press,
Minneapolis, 1935. Pp. xvi + 338, figs. 46, tables 89. Price $2.50.

The subject of land use is now receiving much attention, both from the eco-
nomic and sociological point of view. Most of our Public Domain has passed
into private ownership. We now find ourselves compelled to reexamine our
entire land-use problem and to outline programs and procedures best calculated
to conserve the national interests. We are told by the authors that "The
object of the study on which this volume is based has been to assemble such
facts and to develop their relationship to land use problems. Those who have
participated in the work are not under the illusion that it speaks the final
word on the subject. They look upon it rather as an introductory approach
toward a better understanding of the problem and hope that it will be viewed
as such." The authors also note that "This is the second volume dealing
with the problems of land use to be published by the University of Minnesota
Press. The first, Land Utilization in Minnesota: A State Program for the
Cut-over Lands, published in 1934, was the report of a committee appointed
by the governor and is now out of print."

The book contains 12 chapters and an index. The several chapters are
designated as follows: The Background; Description of the Region; Present
Uses of Land: Natural Areas; The Economic and Social Consequences of Plan-
less Land Use; Present Policies and Programs of Adjustment; Land Classifica-
tion and Zoning; Improved Utilization of Private Forest Lands; Acquisition
and Utilization of Land for Public Purposes; Improved Use of Agricultural
Land; Problems Involved in Moving Farm Families; Adjustments in Local
Government; and Translating Proposals into Effective Programs.

The book is well documented and illustrated, and represents a valuable con-
tribution on the subject of land use.

A Commercial and Economic Geography. By NEIL F. MORRISON. The Ryer-
son Press, Toronto, 1934. Pp. xv + 557, figs. 135.

The major divisions of the book are devoted to: General Principles; Eco-
nomic Products; The Dominion of Canada; and The British Empire. The first
of these consists of seven chapters, designated, respectively: The Influence of
Topography upon Commerce and Industries; The Influence of Climate upon
Commerce and Industries; The Distribution of the World's Population; The

Inter-Relationship of Commerce and Civilization; The Development of Trade Centres; The Development of Transportation and Storage; and Agencies which Hinder Commerce. The second division comprises chapters on: Cereals; Fruits and Vegetables; Sugar, Beverages, Spices and Tobacco; Live Stock, Meats and Dairy Products; Fish; Leather; Textiles; Forest Resources and Industries; Rubber; Power; Coal; Petroleum; Iron; Clay and Clay Products; The Precious Metals; and Other Minerals. In the third division, we find chapters on: Ontario; Quebec; New Brunswick; Nova Scotia; Prince Edward Island; Manitoba; Saskatchewan; Alberta; British Columbia; The Yukon Territory; The Northwest Territories; Canadian Agriculture; The Grain Business; Canada's Forests and Forest Industries; Canada's minerals and Mineral Industries; Canada's Commercial Fisheries; Canadian Furs; Canada's Manufacturing Industries; Canada's Transportation Facilities; and Canada's Trade Relations. In the fourth part, the five chapters are designated, respectively: British Possessions in Europe; British Possessions in Asia; British Possessions in Africa; British Possession in Oceania; and British Possessions in the Americas.

Much useful information has been brought together in this volume. The reader will be able to obtain, as it were, a bird's eye view of a great political and economic domain. He will also recognize that the author has made a successful effort to show that Canada is but a part of a greater empire.

Rural Britain To-Day and To-Morrow. By JAMES A. S. WATSON. Oliver & Boyd, Edinburgh and London, 1934. Pp. xxiii + 161, Illus. 12.

Those who know their rural England will find much pleasure in reading this little book. It is reminiscent of the old and offers inspiration for the new. In the introductory statement by Walter E. Elliot, Minister of Agriculture and Fisheries, we are told that "This is a survey of the country, the countryside and its crops, the countryside and its stock, the countryside and its villages and roads, its schools, its buses and its wireless sets, and all that arises out of them. The countryside as it is cultivated and inhabited is the truest picture of a people."

The book contains 12 chapters, entitled, respectively: The Changing Countryside; Wresting a Living from the North Lands; Fishermen and Farmers in North-East Scotland; Lowland Scotland; From the North to the Midlands; East Yorkshire and the Fens; Women's Institute; Tradition and Experiment in the West; The South-Eastern Counties; East Anglian Industries; The West Country; In Corbett's Day and Now; and Conclusion.

In the concluding paragraph of the book, we find this: "Country life is changing, as farming is changing, in some ways regrettably, but on the whole for the better. The disadvantages of isolation, of long dark winter nights, of gruelling hard work on the land, are gradually being mitigated. We only need to give the countryman a fair reward for his toil in order to make his life happier than it has ever been. And apart from financial anxieties the life on the land is still the best of all lives for men and women to lead."

America's Capacity to Produce. By EDWIN G. NOURSE and associates. The Brookings Institution, Washington, 1934. Pp. xiii + 608, numerous tables and graphs. Price $3.50.

In preparing this volume, the author had the benefit of the collaboration of Frederick C. Tryon, Horace B. Drury, Maurice Leven, Harold G. Moulton, and Cleona Lewis.

The present volume is Publication No. 55 of the Institute of Economics of the Brookings Institute. The following may be quoted from the Foreword: "In the light of existing conditions and current experimentation in the realm of economic organization, the present moment seems opportune to examine anew the foundations of economic progress. Is there adequate reason to suppose that the economic activities of our people could be organized on a sustained level which would permit ample food, adequate clothing, comfortable housing, and a reasonable minimum of education and recreation for all members of society? If so, what is the type of economic organization and, more particularly, what are the ways of conducting the affairs of an economic society so organized which would assure the attaining of such a permanent high standard of material well-being."

The Director's Preface, Foreword, and Introduction fully describe the purpose which lay behind the preparation of the book. Part I deals with raw materials; Part II, with fabrication; and Part III, with services. There are six appendixes and an adequate index. Part I includes six chapters, on: Agriculture; Coal and Coke; Petroleum; Copper and other Non-Ferrous Metals; Cement and other Earth Materials; and Conclusions on Capacity of the Mineral Industries. In Part II, we find chapters on: Manufacturing: General Considerations; Food Products; Textiles and Clothing; Automobiles and Tires; Paper Making, Printing and Publishing; Iron and Steel; Other Manufactures; and Conclusions on Manufacturing Capacity. In Part III, the several chapters comprise: Electric Power Utilities; Transportation; Merchandising; Money and Credit; The National Labor Force; and Conclusions. Appendix A has to do with Methods Used in Adjusting Figures for Agricultural Capital; Appendix B, with Measuring Productive Capacity in Mining; Appendix C, with Note on the Statistics of Rated Capacity of Portland Cement Mills; Appendix D, with Notes on Electric Utility Capacity; Appendix E, with Detailed Labor Data and Computation Methods; and Appendix F, with Statistical Tables.

Like other publications of the Brookings Institution, the present volume is substantial and authoritative. The subject matter is presented in a most interesting way.

Economic Development in Modern Europe. By CLIVE DAY. The Macmillan Company, New York, 1933. Pp. xiv + 447. Price $2.50.

The purpose which prompted the author to write this book is indicated in his statement: "In this sketch of economic development I have attempted

something approaching the form of constitutional history. The reader must look elsewhere for the incidents of the economic narrative, and for a more extended treatment of such particular topics as commerce, credit, and labor. This book aims to present for study the institutions of the two most important branches of production,—agriculture and manufacture, and to suggest their relations to the political and social conditions of the times."

There are in the book 19 chapters, a list of references, and an index. The treatment of the subject matter is indicated by the designations of the chapters, as follows: England in the Eighteenth Century; The Industrial Revolution in England; England in the Nineteenth Century; English Trade and Manufacture, 1873–1914; English Manufacture Since the War; England: Post-War Problems; France to 1789; France: Agriculture; France: Manufacture; Post-War France; Germany; German Manufacture, 1871–1914; Post-War Germany; Russia Before and After Emancipation; Organization of Russian Agriculture; The Russian Peasant; Russian Manufacture: Early Forms; Russian Factory Industry; and The Russian Revolution.

The reader of the book is readily impressed with the significance of the land factor in the economic development of modern Europe as described by the author. In all of the European countries mentioned by him, and in some much more than in others, the volume and character of agricultural commodities produced and their movement into the channels of trade have played a prominent rôle in influencing social as well as economic conditions—national and international. The return from the land has been affected, on its part, by the fertility of the soils in different European countries. The student of land and soil problems, as well as the economist, the sociologist, the historian, and the general reader will find much information of absorbing interest in this book.

Outline of Town and City Planning. By THOMAS ADAMS, With a Foreword by Franklin D. Roosevelt. Russell Sage Foundation, New York, 1935. Pp. 368, illus. 126. Price $3.00.

In the Foreword of the book, President Roosevelt says: "City planning is as old as civilization, but among the most important needs of our modern civilization is the proper regulation of both urban and rural development. It is especially true in America, where urban expansion has been exceptionally rapid and has tended in recent generations toward over-concentration, and where rural industries continue to bulk so large in our national life. Often this has led to blighted areas and serious economic waste. To meet these needs we must exercise more foresight in regulating the development of land.

There is an Author's Preface; an Introduction dealing with: Object of City Planning; Scientific Basis of City Planning; the Art of City Planning; Problems in City Planning; Public Policy and Leadership; and the City Planner and Practice. Part I of the book is designated, "Early Efforts in Town and City Planning"; and Part II, "Modern Phases of Urban Growth and City Planning."

There are four chapters in the first part, entitled, respectively: Ancient City

Planning; City Planning in Europe in the Middle Ages; City Planning During and After the Renaissance Period; and Significance of Early Efforts in City Planning. In Part II, we find the following named chapters: Formative Influences of Modern Civic Growth; Developments in the United States before 1900; New Forces in Urban Growth in the United States; City Planning in the United States between 1900 and 1909; Recent Developments in City Planning in the United States; Town Planning Outside the United States; Aims and Methods of Modern City Planning in America; and the Future of City Planning. The Appendix represents a summary of aspects of city planning problems, and deals with the topics of Engineering; Landscape Architecture; Architecture; Sociology; Economics and Finance; and Law. There are two pages of bibliographical notes and an Index.

This book is a welcome addition to our literature on the subject of town and city planning. It is well balanced, and the story in it is well told.

Food Products, Beverages, Rubber, Tobacco, and Miscellaneous Manufactures Based on Vegetable Products 1917–27. Canadian Department of Trade and Commerce. And *Food Products, Beverages, Rubber, Tobacco and Miscellaneous Manufactures Based on Vegetable Products* 1932. By F. A. ACLAND. Ottawa, 1929 and 1934.

These are statistical reports on the subjects mentioned in the titles. The data have evidently been got together with the usual care and accuracy and will be studied with appreciation by statisticians, economists, agronomists, and others.

Report for 1933 Rothamsted Experimental Station. Harpenden, England.

The report falls into two sections: one dealing with the field work on fertilizer and cultivation problems at Rothamsted, Woburn, and many outside centers in various parts of England; the other summarizing the laboratory investigations, the details of which are to be found in the 52 scientific papers and 29 technical papers published in 1933. Full abstracts of all scientific papers dealing with the laboratory findings are given in the report. Agricultural advisers, teachers, students, and others interested in the technical advancement of farming will find the results of the year's experiments at the Rothamsted Experimental Station as set forth in this report of considerable importance.

JACOB G. LIPMAN.

AUTHOR INDEX

SUBJECT INDEX